Physiological Responses to Exercise in Omani Children with Sickle Cell Disease

Al-Kitani, Mahfoodha

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PHYSIOLOGICAL RESPONSES TO EXERCISE IN
OMANI CHILDREN WITH SICKLE CELL DISEASE

Mahfoodha Al. Kitani

A thesis submitted for the degree of Doctor of Philosophy

University of Bath

School for Health

January 2013

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ABSTRACT

According to the national survey of genetic blood disorders, the prevalence of haemoglobinopathies in Oman is 9.5% with Sickle Cell Disease (SCD) and Sickle Cell Trait (SCT) representing two major public health concerns and having great impact on individuals’ lives as well as on society (Al-Riyami et al., 2001).

Complications related to SCD arise owing to ischemic tissue injury and may result in organ dysfunction and premature death. Patients with SCD often experience painful crises (vaso-occlusion), renal disease, acute chest syndrome (ACS) and other life-threatening conditions. Physical education teachers tend to exclude children with SCD from PE classes due to their health status. It is currently unknown whether exercise might have beneficial, adverse or no effect in children and adults with SCD. Consequently, the recommendation of exercise for children and adults with SCD is rare. accordingly, there were three objectives to the work within this thesis:

First, to investigate physical fitness markers in SCD and SCT and compare them to normal healthy children. With no reference data available, the aim of the first study was to provide a general idea of this population’s physical fitness parameters. The results suggest that children with SCT had similar anthropometric measurements, physical fitness and exercise responses to normal healthy children. In contrast, SCD children who were shorter and had lower body mass, higher fat mass and lower physical fitness than SCT and normal healthy children. In addition, children with SCD exhibited higher heart
rate and blood lactate responses in response to exercise than SCT children and normal healthy children.

The second study was designed to determine cardiovascular responses to exercise in children with SCD and SCT and to compare them with those of normal healthy children from the same age ranges. Normal healthy children had a significantly higher estimated maximal oxygen uptake (VO$_2$max) than SCT and SCD children ($P < 0.05$), with SCD children achieving the lowest VO$_2$max. The mean heart rate in SCD and SCT was significantly higher during sub-maximal exercise than in normal healthy children ($P < 0.05$). White blood cell (WBC) count was also higher ($P < 0.05$) in the SCD group than in the other two groups, which is suggestive of a chronic inflammatory state.

The third study aimed to determine whether a single bout of exercise elicits changes in interleukin-6 (IL-6) concentrations in children with SCD. Children with SCD exhibited higher baseline IL-6 concentrations than the normal healthy children ($P < 0.05$), suggesting a persistent inflammatory state. However, the exercise bout did not elicit a significant change in IL-6 concentrations in either normal healthy or SCD children.

The final study investigated the effect of an acute bout of exercise on postprandial changes in triacylglycerides (TAG), glucose, insulin and total cholesterol. Postprandial TAG concentrations were reduced in the exercise trial ($P < 0.05$). The postprandial glucose and insulin responses were also reduced in the exercise trial ($P < 0.05$).

In conclusion, the work in this thesis suggests that Omani children with SCD have lower physical fitness than normal healthy children, and that exercise might have
beneficial effects on this population. The lower physical fitness of SCD children is associated with altered body composition and lower oxygen-carrying capacity of the blood, as these children had shorter stature, lower body mass, higher fat mass and exhibited lower VO$_2$max. The potential benefit of exercise for this population is demonstrated by the alterations of postprandial lipaemia after an acute bout of exercise. Higher TAG has been associated with increased incidence of vaso-occlusive events in subjects with SCD, and exercise lowered postprandial TAG concentrations in children with SCD.
ACKNOWLEDGEMENTS

I would like to thank DR. Keith Stokes for agreeing to be my academic advisor. His advice and guidance have been very much appreciated. I would also like to thank all those who helped and supported me the whole way (my Mum, Dad and husband). A special thanks to my youngest sister, Salwa, for her support and assistance.

I must not forget to express my appreciation to all of the staff working at Sultan Qaboos University Hospital (SQUH) and Sur Regional Hospital (SRH). This research would not have been accomplished without the collaboration of the young participants, parents and the SQUH and SRH administrations. I would like to thank them all for their great contribution and patience.

My grateful thanks to all of my colleagues at the University of Bath who were willing to help me at any moment.
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<tr>
<td>aBMD</td>
<td>Areal bone mineral density</td>
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<tr>
<td>ACS</td>
<td>Acute chest syndrome</td>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>AT</td>
<td>Anaerobic threshold</td>
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<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
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<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>Bp</td>
<td>Blood pressure</td>
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<tr>
<td>Bpm</td>
<td>Beats per minute</td>
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<tr>
<td>BSA</td>
<td>Body surface area</td>
</tr>
<tr>
<td>CBC</td>
<td>Complete blood count</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>DEXA</td>
<td>Dual energy x-ray absorptiometry</td>
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<tr>
<td>DPG</td>
<td>Diphosphoglycrol</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>ET-1</td>
<td>Endothelin-1</td>
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<tr>
<td>FFA</td>
<td>Free fatty acids</td>
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<tr>
<td>FFM</td>
<td>Fat-free mass</td>
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<tr>
<td>GH</td>
<td>Growth hormone</td>
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<tr>
<td>Hb</td>
<td>Haemoglobin</td>
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<tr>
<td>HbAA</td>
<td>Normal haemoglobin</td>
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<tr>
<td>HbAS</td>
<td>Heterozygous sickle cell</td>
</tr>
<tr>
<td>HbSS</td>
<td>Homozygous sickle cell</td>
</tr>
<tr>
<td>Hct</td>
<td>Haematocrit</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin 6</td>
</tr>
<tr>
<td>IL-10</td>
<td>Interleukin 10</td>
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Kcal  Kilocalorie
LDH  Lactate dehydrogenase
LDL  Low density lipoprotein
LPL  Lipoprotein lipase
LTEQ Leisure Time Exercise Questionnaire
MCHC Mean corpuscular haemoglobin concentration
MFT Multi-stage fitness test
mmol.l\(^{-1}\) millimole per litre
NO Nitric oxide
OFTT Oral fat tolerance test
O\(_2\) Oxygen
PA Physical activity
PDH Pyruvate dehydrogenase
PE Physical education
PHV Age at high peak velocity
pO\(_2\) Partial pressure of oxygen
<table>
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<tr>
<th>Abbreviation</th>
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<tr>
<td>PPL</td>
<td>Postprandial lipaemia</td>
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<tr>
<td>RBC</td>
<td>Red blood cell</td>
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<td>SCD</td>
<td>Sickle cell disease</td>
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<td>SCT</td>
<td>Sickle cell trait</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical package for the social sciences</td>
</tr>
<tr>
<td>SQUH</td>
<td>Sultan Qaboos University Hospital</td>
</tr>
<tr>
<td>SRH</td>
<td>Sur Regional Hospital</td>
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<tr>
<td>TAG</td>
<td>Triacylglyceride</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour Necrosis Factor-α</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very low density lipoprotein</td>
</tr>
<tr>
<td>VO2max</td>
<td>Maximal oxygen uptake</td>
</tr>
<tr>
<td>WBC</td>
<td>White blood cell</td>
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<td>WHO</td>
<td>World Health Organisation</td>
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INTRODUCTION

Sickle cell disease (SCD) and sickle cell trait (SCT) are two major public health concerns in Oman that have a great impact on the affected individual and society as a whole. The prevalence of total haemoglobinopathies in Oman is 9.5% according to the national survey of genetic blood disorders in Oman (Al-Riyami & Ebrahim, 2003). The results of this national survey revealed that 7% of Omani children have sickle cell, 2% have β-thalassaemia and 0.2% – 0.7% are homozygous for sickle cell and β-thalassaemia. Although the distribution of these haemoglobinopathies varies among the different Omani regions, the survey revealed that half of all Omani children under five years of age were anaemic. Mild anaemia was predominant (46%), while moderate and severe anaemia were found in 4% and 0.2%, respectively (Al-Riyami & Ebrahim, 2003). Moreover, the status of anaemia among these children improved with age; mild anaemia was evident in 65% of children aged between 0 ≤ 1 and then decreased with age, but was still present in 30% at the age of 4–5 years.

Generally, there is a correlation between the existence of malaria and the presence of sickle cell genes; whenever malaria is found in a region; genetic blood disorder genes are found. It is well known that the frequency of HbSS is at its highest in tropical and subtropical areas. For example, the frequency of HbSS exceeds 20% in some African countries (Allison, 2009). The sickle cell gene occurs in Middle Eastern countries such as Saudi Arabia, Bahrain, Lebanon and Oman (El Hazmi et al., 2011). Complications related to SCD due to ischaemic tissue injury result in organ dysfunction and premature
death. Patients with sickle cell often experience painful crises (vaso-occlusion), renal disease, ACS and other life-threatening conditions (Mitchell, 2000; Platt et al., 1994; Young et al., 2006).

Sickle cell disease is an inherited haemolytic blood disease. It is caused by a point mutation. The mutation causes a substitution of the amino acid glutamine with valine at position 6 in the Beta chain of the globin molecule in haemoglobin (Hb) (Ferrone, 2004). Haemoglobin is the protein that gives the red blood cell (RBC) its red colour and carries oxygen (Griffin et al., 1988). The RBCs that contain normal Hb are round and move easily through the blood vessels, while the RBCs that contain the abnormal sickle Hb become distorted and are shaped like a sickle (Griffin et al., 1988). The sickle RBC is sticky, fragile and rigid and may stick in the circulation, causing blockage of the blood flow. In addition, the abnormal Hb has a decreased affinity to oxygen (Steinberg, 1999).

There are two levels of severity of sickle cell anaemia: SCD and SCT. Sickle cell disease refers to a homozygous mutation in the gene (HbSS), while SCT refers to a heterozygous mutation (HbAS). Sickle cell disease is more severe and may cause severe complications and even death, while SCT has fewer complications (Min-Oo & Gros, 2005). Individuals SCD usually die at an early age due to severe blood clotting. Individuals with SCT have enough Hb A to live a relatively normal life, and blood clotting is less severe (Min-Oo & Gros, 2005). The rate of Hb S aggregation and cell sickling depends on several factors, such as blood flow, the concentration of Hb in the blood cells, vigorous exercise, infection and thermal strain (Bergeron et al., 2004; Ferrone, 2004).
A review of the literature revealed little data on exercise, health status, body composition and physical fitness in children and adults with SCD. Physical education (PE) teachers tend to exclude children with SCD from PE classes due to their health status. It is currently unknown whether exercise might have beneficial, adverse or no effects on children and adults with SCD. Consequently, the recommendation of exercise for children and adults with SCD is rare, the effectiveness of regular exercise on children or fitness level and health is still not clear, and how much and for how long children with SCD can exercise is also unknown.

This research will provide new information concerning the importance of exercise for fitness and well-being in children with SCD. It is a first step in the assessment of the role of exercise for children with SCD. The first study will investigate the physical fitness of Omani children with SCD and SCT. Then the cardiovascular responses to exercise in SCD and SCT will be investigated in comparison to normal healthy Omani children. The final two studies will examine the effect of an acute bout of exercise on IL-6 concentrations and the effect of exercise on postprandial lipaemia in this population.
1. LITERATURE REVIEW

1.1 Sickle Cell Disease (SCD)

Sickle cell disease is an inherited haemolytic blood disease. It affects a protein inside the RBCs called Hb. This protein gives the RBC its red colour and also carries oxygen from the lungs to other parts of the body (Griffin et al., 1988). Red blood cells that contain normal Hb are round and move easily through the blood vessels. In contrast, RBCs that contain the abnormal sickle Hb become distorted and shaped like a sickle (Griffin et al., 1988) (see Figure 1.1). The sickle RBC is sticky, fragile and rigid. It may stick in the circulation and block blood flow to tissues. Sickle cell disease follows the Mendelian inheritance mode and affects many races, countries and ethnic groups throughout the world. There are two levels of severity of sickle cell anaemia: SCD and SCT (Min-Oo & Gros, 2005). Individuals with two S Hb alleles are said to have SCD. These individuals usually die at an early age due to severe blood clotting. The other group that has one normal Hb allele and one sickle allele are said to have SCT. This group has enough HbA to live a relatively normal life, since blood clotting is less severe (Min-Oo & Gros, 2005).

Evidence suggests an association between the sickle cell gene and malaria. Malaria is distributed across the southern Mediterranean, the Middle East, sub-Saharan Africa, India, south Asia and northern Australia. In these areas, there is a relatively high percentage of the sickle cell allele within the gene pool ancestry (Steensma et al., 2001). Sickle cell exceeds a frequency of 10% in Africa and is only advantageous in regions
where the incidence of malaria is high. The development of the malaria parasite plasmodium flaciporum is disrupted by the sickling of the RBCs. Since the natural habitat for plasmodium flaciporum is the normal blood cell with HbAA, the sickle cell gene provides protection against malaria for individuals with both HbAS and HbSS (Aliyu et al., 2008). The sickled cell may be prematurely removed by the spleen or shed nutrients, like potassium, that the parasite needs to survive; therefore, the parasite dies (Min-Oo & Gros, 2005). Because the malaria parasite cannot live in sickle cells, individuals with S Hb are resistant to malaria (Min-Oo & Gros, 2005). When compared, individuals with HbAA have a higher parasitic load than those with HbAS and HbSS. As individuals with HbSS do not live to a reproductive age, and HbAA individuals are killed by the parasite, individuals with HbAS become the predominant in the population, as they have enough HbA to maintain relatively sufficient blood function and enough HbS to retain resistance to malaria (Min-Oo & Gros, 2005), giving rise to the S gene in the population (Aliyu et al., 2008).
1.1.1 Sickle Cell Trait (SCT)

Sickle cell trait is not a disease, but a condition in which a person has both normal and sickle Hb in the red cells as a result of inheriting a normal Hb allele and an allele for sickle Hb (Bar-Or & Rowland, 2004). The destruction of the Hb molecule structure and its dysfunctions do not normally cause abnormalities in subjects with SCT. Some researchers, however, have reported that certain abnormalities may occur with increased frequency in subjects with SCT, such as haematuria (blood in the urine) (Le Gallais et al.,
1996). Furthermore, although the lower oxygen affinity of HbS does not cause major abnormalities in HbAS carriers, certain abnormalities do occur, such as rhabdomyolsis, splenic syndrome and tissue necrosis (Marline et al., 2005). Sickle cell trait cannot develop into SCD, but the sickle gene may pass to the next generation. Table 1.1 summarizes the differences between SCT and SCD. If both parents have SCT or another Hb trait, there is a 25% chance that their child will have SCD. Rehan (1981) stated that children with SCT, compared with normal healthy children, follow the same growth patterns. Nutritional and environmental factors may affect the presence of complications as well as growth in SCT (Barden et al., 2002).
Table 1.1: Differences between sickle cell disease and sickle cell trait

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<td><strong>HbAA:</strong></td>
<td>Normal haemoglobin refers to the whole molecule</td>
</tr>
<tr>
<td><strong>HbSS:</strong></td>
<td>Sickle cell haemoglobin (homozygous mutant)</td>
</tr>
<tr>
<td><strong>Hbα:</strong></td>
<td>Gene for normal haemoglobin alpha chain</td>
</tr>
<tr>
<td><strong>Hbβ:</strong></td>
<td>Gene for normal haemoglobin beta chain</td>
</tr>
<tr>
<td><strong>HbβS:</strong></td>
<td>Gene for mutant haemoglobin beta chain, the sickle cell haemoglobin</td>
</tr>
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<td><strong>Structure of Normal Haemoglobin Molecule (HbAA):</strong></td>
<td>2 alpha and 2 beta chains</td>
</tr>
<tr>
<td><strong>Structure of Sickle Cell Disease Molecule (HbSS):</strong></td>
<td>2 alpha and 2 s chains</td>
</tr>
<tr>
<td><strong>Composition of Haemoglobin in Persons with Sickle Cell Disease (HbSS):</strong></td>
<td>All haemoglobin molecules consist of 2 alpha and 2 s chains</td>
</tr>
<tr>
<td><strong>Composition of Haemoglobin in Persons with Sickle Cell Trait (HbAS):</strong></td>
<td>Half of their haemoglobin molecules consist of 2 alpha and 2 beta chains, and half consist of 2 alpha and 2 s chains</td>
</tr>
</tbody>
</table>

(Source: [http://www.nslc.wustl.edu/sicklecell/part2/html](http://www.nslc.wustl.edu/sicklecell/part2/html)).
Structure and function of red blood cells

1.1.2 Erythrocyte Structure

Erythrocytes, or RBCs, are the most abundant cell type in the blood. A microlitre of blood contains, on average, about 5 million RBCs, compared with only 4,000–11,000 WBCs and 20,000–500,000 platelets (Hoffbrand et al., 2001). Consequently, RBCs are the major factor contributing to blood viscosity. Mature mammalian RBCs are biconcave disks with a diameter of about 7 μm, a thickness of 2.5 μm at the periphery, 1 μm in the centre and a volume of 85–91μm³ (Hoffbrand et al., 2001). Red blood cells lack a nucleus and membranous organelles, and their cytoplasm is full of the red, oxygen-carrying pigment Hb (97% of a RBCs dry mass is Hb). Haemoglobin consists of iron-containing haem groups conjugated to four polypeptide chains that form the globin protein (Hoffbrand et al., 2001).

1.1.3 Function

The primary function of RBCs is to facilitate oxygen and carbon dioxide transport between the lungs and respiring cells (Hoffbrand et al., 2001). The RBC is a perfect example of complementarities of structure and function. Each of the structural characteristics of a RBC contributes to its gas transport functions: its biconcave shape creates a huge surface area to volume ratio, which facilitates gas exchange across the cell membrane (Hoffbrand et al., 2001). The biconcave shape of the cell is maintained by a complex cytoskeleton, composed of protein filaments such as spectrin. Despite this cytoskeleton, RBCs are remarkably flexible, which allows them to change shape as they
squeeze through the narrow capillaries of the circulation (Sherwood, 2004). Their structure also allows them to modify their shape in response to osmotic changes in the blood. A cell placed in slightly hypertonic solution will swell and form a sphere without disruption of its membrane integrity (Young et al., 2006). In hypotonic media, the cells shrink and the plasma membrane pulls tight against the cytoskeleton. Another property of the RBC that facilitates gas transport is the percentage of Hb within the cell (Sherwood, 2004). Ninety-seven per cent of a RBCs dry mass is Hb, which maximizes the oxygen-carrying ability of the cells. In the lungs, Hb preferentially binds oxygen, forming oxyhaemoglobin. In the respiring tissues, the process is reversed: Oxyhaemoglobin releases oxygen as it detaches from the iron and forms deoxyhaemoglobin, or reduced Hb (Hsia, 1998). About 20% of the carbon dioxide transported in the blood combines with Hb (forming carbaminohaemoglobin), but it binds to the amino acids of globin rather than with the haem group (Young et al., 2006).

Because RBCs lack mitochondria and generate Adenosine triphosphate (ATP) anaerobically, they do not consume the oxygen that they are transporting, making them even more efficient oxygen transporters. A secondary consequence of having no nucleus or endoplasmic reticulum is that a mature RBC is unable to carry out protein synthesis. The cell is unable to make new enzymes or renew its membrane components. This inability leads to an increasing loss of membrane flexibility, making older cells more fragile and likely to break (Young et al., 2006). Therefore, their life span is short. The RBCs can survive for 120 days on average and must be replaced at an average rate of 2 to 3 million cells per second (Young et al., 2006). Because RBCs cannot divide to replenish
their own number, old ruptured cells are replaced by new ones that are produced in the bone marrow (Sherwood, 2004). The bone marrow is a highly cellular tissue that fills the intracellular cavities of the bones. There are two types of bone marrow: red and yellow. However, only red bone marrow is capable of producing blood cells (Sherwood, 2004). The process of manufacturing new RBCs from the bone marrow is called erythropoiesis. Erythropoiesis is regulated by an enzyme called erythropoietin that is mainly produced by the kidneys in response to a reduced oxygen carrying capacity of blood (Sherwood, 2004). Most old RBCs meet their demise in the spleen, because its narrow, winding capillary network is a tight fit for the fragile cells (Sherwood, 2004). In addition to removing most of the old RBCs from the circulation, the spleen has a limited ability to store healthy RBCs in its pulpy interior (Sherwood, 2004).

Table 1.2: Normal values of blood indices in adults

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>13.5 – 17.5</td>
<td>11.5 – 15.5</td>
</tr>
<tr>
<td>Haematocrit %</td>
<td>40 – 52</td>
<td>36 – 48</td>
</tr>
<tr>
<td>Red cell count (x10^{12}/l)</td>
<td>4.5 – 6.5</td>
<td>3.9 – 5.6</td>
</tr>
<tr>
<td>Mean cell haemoglobin (pg)</td>
<td>27 – 34</td>
<td>27 – 34</td>
</tr>
<tr>
<td>Mean cell volume (fl)</td>
<td>80 – 95</td>
<td>80 – 95</td>
</tr>
<tr>
<td>Mean cell haemoglobin concentration (g/dl)</td>
<td>30 – 35</td>
<td>30 – 35</td>
</tr>
<tr>
<td>Reticulocyte count (x10^9/l)</td>
<td>25 – 125</td>
<td>25 – 125</td>
</tr>
</tbody>
</table>

In children, normal Hb values are: newborn, 15.0 – 12.0 g/dl; 3 months, 9.5 – 12.5 g/dl; 1 year to puberty, 11.0 – 13.5 g/dl. Source: (Hoffbrand et al., 2001, p. 20).
1.1.4 HbSS RBC

Red cells containing HbSS have a much wider distribution of volumes and densities than red cells containing HbAA (Table 1.2). Red blood cells containing HbSS tend to be very dense, compared to normal RBC (Bookchin & Lew, 1996). Blood samples from a population carrying HbSS contain very light cells, most of which are reticulocytes, along with cells with densities greater than the densest normal RBC. The increased density in some HbSS cells is due to an increase in mean corpuscular Hb concentration (MCHC) resulting from cation loss and cellular dehydration (Joiner et al., 1993). De-oxygenation is required for cation loss, dehydration and the formation of dense cells. The dense cell fractions observed in a given HbSS population blood contain many irreversibly sickled cells that can retain their elongated sickled shape even in oxygenated blood (Bookchin & Lew, 1996). The number of irreversibly sickled cells can vary from 10% in one patient to as much as 60% in another patient (Baker et al., 1998). Also, the number of irreversibly sickled cells in a blood volume can vary in any given patient. Irreversibly sickled cells are dehydrated, with a decreased amount of cellular water, an increased MCHC, altered monovalent cation content, increased sodium and very low potassium (Hebbel, 1991).

1.1.5 Pathogenesis of sickle cell disease

Sickle cell disease is caused by a point mutation that substitutes the amino acid glutamine with valine at position 6 in the Beta chain of the globin molecule in Hb. Glutamic acid is a charged hydrophilic molecule that exists at the exterior surface of Hb,
while valine is a neutral hydrophobic molecule that exists within the core of the protein (Ferrone, 2004). This substitution results in the formation of a sticky patch, called the hydrophobic pocket, on the surface of the Beta chain of globin (Ferrone, 2004). During the release of oxygen from Hb (de-oxygenation) in the capillaries, the hydrophobic pocket causes the polymerization of S-Hb molecules into long fibres, forming a complex helical molecule within the RBC (Steinberg, 1999). The formation of these fibres is dependent in the presence of valine at the beta-6 position to bind another beta chain of another Hb molecule. The polymerization process causes the RBC to undergo morphological changes from the flexible disc shape to the rigid sickle shape (Steinberg, 1999) (Figure 1.2). The polymers consist of 14-strand fibres that distort the shape of the erythrocyte membrane, changing its normal biconcave disc shape to a number of other shapes, such as granular, holly-leaf and the classic sickle shape. The whole process causes a decrease in the affinity of the Hb to oxygen, whether by the substitution of an active amino acid with another, less active amino acid or by the reduction of the binding surface area via Hb polymerization and RBC shape changes (Steinberg, 1999). When the HbS erythrocyte (reversibly sickle cells) is re-oxygenated, they resume the normal shape. The repeated erythrocyte oxygenated and de-oxygenated configuration cycles result in permanent damage to the cell membrane leading to irreversibly sickled cell which is then destroyed or haemolysed. The haemolysis shortens the lifespan of the erythrocytes as the normal erythrocyte lifespan is around 120 days, while the sickled erythrocyte lifespan ranges between 10 and 14 days. This whole process leads to chronic haemolytic anaemia (McCurdy & Sherman, 1978; Ohnishi et al., 1986).
In the microcirculation, the normal blood cells move without problems through the smallest capillaries, but in SCD, when the red cells release oxygen they change shape to the sickle shape and become rigid (Young et al., 2006). As a result, they fail to move smoothly along the blood vessels, blocking the blood flow to the nearby tissues. This blocking of blood flow, magnified by the low affinity of Hb to oxygen, may cause tissue hypoxia (low O₂ supply), which can cause tissue damage and pain (Young et al., 2006).

Figure 1.2: The polymerization process of Hb SS

(Adapted from Bessa et al. (1992)).
1.1.6 Types and symptoms of sickle cell anaemia

There are several types of sickle cell anaemia. Sickle cell disease is the most common form, where the individual inherits two sickle cell alleles. Another type is sickle- C disease, where the person inherits one sickle cell allele and one allele for another abnormal type of Hb called HbC. A third type is S beta-zero thalassemia (one sickle cell allele and one allele for a type of thalassaemia) (Young et al., 2006). All forms of sickle cell anaemia are characterized by production of HbS, chronic haemolytic anaemia, acute and chronic ischaemic tissue damage owing to vascular obstruction (Young et al., 2006).

Symptoms of the disease include painful crises resulting from acute vaso-occlusive episodes. Painful crises are the primary symptom of SCD in children and adults. The pain may be caused by small blood vessel blockages that prevent oxygen from reaching tissues (Habibi et al., 2004). The crises are unpredictable and can affect any area of the body. The chest, abdomen and bones are frequently the most affected sites. The frequency and duration of the pain can vary. Crises may be separated by more than a year or possibly only by weeks, and they can last from hours to weeks (de Montalembert, 2004). Hand-foot syndrome is one particular type of painful crisis and is often the first sign of SCD in an infant. Symptoms include pain and swelling in the hands and feet, possibly accompanied by a fever. Hand-foot syndrome typically occurs only during the first four years of life (de Montalembert, 2004). There is some evidence that cold temperatures or infection can cause painful crises, but most crises occur for
unknown reasons (de Montalembert, 2004). Several complications are associated with SCD, including an enlarged spleen and the associated increased risk of infections, delayed growth, increased stroke risk and ACS, whereby sickle cells block pulmonary blood vessels.

Sickle cells can block blood flow through the spleen and cause damage to the organ. In infants and young children with SCD, the spleen is usually enlarged. After repeated blood vessel blockage, the spleen usually atrophies by late childhood (Mitchell, 2000). Damage to the spleen can have a negative impact on the immune system, leaving individuals with SCD, especially infants and young children, open to infections.

The energy demands on the bone marrow for RBC production compete with the demands of a growing body (Silva & Viana, 2002). Children with SCD have delayed growth and reach puberty at a later age than normal. By early adulthood, they catch up on growth and attain normal height; however, their weight typically remains below average (Thomas et al., 2000). Studies in exercise and SCD have demonstrated the relationship between SCD and the delay in sexual and skeletal maturation in children. There is an increasing rate of erythrocytes production, cardiac workload and rate of protein synthesis and catabolism, resulting in a greater energy and protein requirement in children with SCD, compared with normal healthy children (Barden et al., 2000).

Blockage of blood vessels in the brain may have particularly dangerous effects and can be fatal. Oxygen is needed for all areas of the brain, and a lack of oxygen delivery may result in loss of control of the functions associated with the affected areas of
the brain (Kirkham & DeBaun, 2004). Sometimes this loss is permanent. Children with SCD are at high risk for ischemic stroke and transient ischemic attacks. The aetiology is usually intracranial arterial dysfunctions involving mainly the carotid and the cerebral arteries. Children with SCD between the ages of 1 and 15 have the highest risk of suffering a stroke (Kirkham & DeBaun, 2004). Approximately two-thirds of children who have a stroke will have at least one more (Kirkham & DeBaun, 2004). Although it is expected that the ischemic brain complications are due to the sickling of blood leading to clots that occlude the arteries, it has been shown that the main mechanisms involve pathology of the arterial walls, such as stenosis (Kirkham & DeBaun, 2004).

One of the main features of SCD that can occur at any age is acute chest syndrome, which is caused by sickle cells blocking the small blood vessels of the lungs (Pianosi et al., 1991). This blockage is complicated by accompanying problems such as infection of the blood in the lungs. Affected persons experience chest pain, fever, cough and shortness of breath (Pianosi et al., 1991). Repeated attacks can lead to permanent damage to the lung.

All of these features of SCD vary with age. For example, in early childhood and adolescence, the most common fatal complication is infections, most predominantly lung infections (Leikin et al., 1989; Platt et al., 1994), while in adulthood the most common fatal complication is chronic organ damage, such as renal or cardiac failure and cerebrovascular accident (Platt et al., 1994).
1.1.7 Factors affecting sickling

The rate at which Hb S aggregation and cell sickling occur depends on several factors, such as the blood flow and the concentration of Hb in the blood cells. Therefore, if blood flows at a normal rate, Hb S is re-oxygenated in the lungs before it has a chance to aggregate (Ferrone, 2004). The concentration of HbSS in the erythrocyte is also of great importance, with higher concentrations of HbSS leading to more rapid polymerization (Schnog et al., 2004). The concentration of Hb within the RBCs is influenced by the individual’s hydration level (the amount of water contained in the cells) (Bergeron et al., 2004). Therefore, if a person becomes dehydrated, Hb becomes more concentrated in the RBCs. In this situation, Hb S has a greater tendency to clump together and induce sickle cell formation. Decreases in blood pH (reducing the affinity of Hb for O₂), de-oxygenation and an increase in body temperature are also contributing factors to the intercellular polymerization of HbSS (Bunn, 1997; Kaul et al., 1996). Vigorous exercise, infection and thermal strain may induce sufficient hyperthermia (heat stroke), hyperosmolality, acidosis and red blood dehydration, leading to significant erythrocyte sickling (Bergeron et al., 2004).

1.1.8 Treatment of sickle cell disease

Hydroxyurea, blood transfusion and bone marrow transplant are among the main options for treatment of sickle cell anaemia. Hydroxyurea, was shown to decrease the number and severity of vaso-occlusive attacks (Charache et al., 1995) and possibly increase survival time (Steinberg et al., 2003). As hydroxyurea had previously been used
as a chemotherapy agent, there is a concern that long-term use may be harmful, but it has been shown to be likely that the benefits outweigh the risks (Platt, 2008). Blood transfusion therapy is often used in the management of SCD in acute cases and to prevent complications by decreasing the number of RBCs that can sickle by adding normal RBCs (Drasar et al., 2011). Bone marrow transplants have proven to be effective in children (Walters et al., 1996).

Some studies suggest that SCD subjects might clinically benefit from exercise therapy. Alcorn et al. (1984) demonstrated that exercise therapy consisting of moderate strength and endurance exercise for 10 to 30 minutes duration may reduce the length of hospitalization and incidence of vaso-occlusive painful crisis in children with SCD. Recently, Tinti et al. (2010) indicated that exercise successfully reduced pain episodes and increased respiratory muscles strength of a SCD patient. The exercise therapy in this study also involved aquatic rehabilitation, aerobic exercise and relaxation. However, clinicians are hesitant to recommend physical activity for patients with SCD as exercise and physical activity may induce vast metabolic alterations that may precipitate vaso-occlusive crises.

1.1.9 Pathophysiological abnormalities of SCD that may influence exercise tolerance

Exercise studies in the sickle cell population are sometimes considered risky and difficult to perform, especially in children. Unsupervised, exercise programmes may lead to regional hypoxemia, acidosis, dehydration, changes in blood osmolality and haemolysis (Bergeron et al., 2004; Moheeb et al., 2007; Platt, 1982; Pyne, 1994). These
conditions may cause sickling, microvascular occlusions and painful crises in the SCD population. It is important to understand the factors that may limit exercise performance in SCD. These factors possibly increase or decrease the risk of sickle cell–related complications. Previous research has pointed to the pathophysiological abnormalities associated with SCD that may affect exercise performance in individuals with SCD, such as anaemia, pulmonary dysfunction, cardiovascular dysfunction and chronic inflammation.

**Anaemia:** As mentioned previously, children with SCD have an increase in erythrocyte destruction (haemolysis). Therefore, they have a greater chance of developing chronic anaemia. A decrease in erythrocyte circulation causes a decrease in oxygen delivery to tissues. According to the oxygen dissociation curve, SCD increases oxygen unloading that occurs at a given partial pressure (pO2) to maintain sufficient oxygen delivery to the tissues (see Figure 1.3). The increase in oxygen unloading due to intracellular polymerization of HbS, increase in 2,3 diphosphoglycrol (DPG) concentrations, and decreased pH cause the oxygen dissociation curve to shift to the right (Charache et al., 1970). Shifting the curve to the right allows more oxygen to be released for a given decrease in pH, which allows more oxygen to be freed from Hb (Pianosi et al., 1993) (see Figure 1.3). In addition, increased respiratory rate and cardiac output help to maintain oxygen delivery to the tissue.

During exercise, there is an increase in body demand of oxygen, owing to the increased metabolic rate and working muscle consumption of oxygen. In SCD, oxygen-
carrying capacity of blood is reduced due to low Hb concentrations (Anthi et al., 2007),
given that oxygen delivery is a limiting factor for VO₂max. The lower VO₂max results in
a shift to the anaerobic metabolism leading to lactic acid accumulation and an early
increase in heart rate and blood pressure (Haywood, 2009). In addition, chronic anaemia
in SCD population may result in structural and functional adaptations of the vasculature,
such as abnormal vascular tone and an activated, adhesive endothelium that results in
lowered blood flow to tissues lowering their exercise capacity (Kato et al., 2009).

Figure 1.3: Haemoglobin dissociation curve in SCD, SCT and normal healthy subjects

Source: adapted from Becklake et al. (1955)
**Pulmonary dysfunction:** Pulmonary complications in SCD are a known cause for acute and chronic morbidity (Hijazi *et al.*, 2005). Acute chest syndrome in SCD is the most threatening complications of pulmonary function. Callahan *et al.* (2002) documented that ACS is a great risk factor associated with the development of progressive pulmonary dysfunction in SCD patients. Acute chest syndrome episodes may lead to chronic lung disease, including pulmonary hypertension, interstitial fibrosis and cor pulmonale that are clinically characterized by progressive disabling dyspnea, hypoxemia and chest pain (Kirkpatrick & Haynes, 1994). The mechanism involves acute lung injury; this injury is caused by multiple external insults. These insults are believed to involve blockage of the pulmonary microvasculature by the sickled erythrocytes, resulting in infarction of the pulmonary parenchyma, macrovascular pulmonary embolism and infections (Weil *et al.*, 1993; Vichinsky & Styles, 1996). Moreover, SCD patients have growth abnormalities that may lead to smaller chest diameter which, in return, affect lung volume. Knight–Madden *et al.* (2005) stated that SCD children with asthma are more likely to suffer ACS.

Exercise is rarely limited by pulmonary causes in normal subjects as they maintain a substantial breathing reserve despite the rise in minute ventilation. Callahan *et al.* (2002) studied factors contributing to exercise intolerance in SCD women during maximal cardiopulmonary exercise and concluded that the mechanisms contributing to the abnormalities in pulmonary circulation in SCD included pulmonary vascular disease that developed as a complication of the ACS. Recurrent episodes of ACS can lead to chronic lung disease, which in return causes gas exchange abnormalities during exercise.
In addition, distorted pulmonary vasoregulation owing to the presence of anaemia caused alterations in pulmonary nitric oxide (NO) and loss of hypoxic pulmonary vasoregulation (Callahan et al., 2002). These alterations may influence and limit pulmonary vasodilatation during exercise in SCD (Callahan et al., 2002). It has been indicated that patients with SCD can achieved only 35% of endurance exercise work achieved by patients with normal Hb and that their minute ventilation is significantly lowered (Oyono-Enguelle et al., 2000).

In SCD children, it has been documented that there is an increased ventilatory response to exercise. Several factors contribute to the higher ventilation rate of SCD children, including low Hb levels and increased alveolar dead space owing to the repeated lung injuries (Pianosi et al., 1991). Children with SCD tended to hyperventilate and develop a state of reduced carbon dioxide in the blood due to rapid breathing even in the presence of elevated dead space. However, even with a low VO2 max in SCD children, the ventilatory anaerobic threshold occurs at a similar percent VO2 max as in normal healthy children indicating a pulmonary contribution to the exercise limitation in this population (Pianosi et al., 1991).

**Cardiovascular dysfunction:** To adjust for the chronic anaemia of SCD, patients with SCD show an increase in cardiac output and respiratory rate at rest to maintain oxygen delivery to tissue. The increase in cardiac output is associated with increases in heart rate and stroke volume (Batra et al., 2002). To compensate for the increase in stroke volume, there is an increase in ventricular dimension in SCD patients. Lester and Sodt (1990) found that patients with SCD had elevated left ventricular, right ventricular, left
arterial champer dimensions and increased inter-ventricular wall thickness. Abnormalities in cardiovascular function were strongly related to the degree of anaemia and percentage of Hb S, but not to the same extent with age. These findings align those reported by Covitz et al. (1995) that suggested age as an important factor in the development of cardiovascular changes in SCD.

At rest, children with SCD compensate for low oxygen and carbon dioxide-carrying capacity by increasing their cardiac output and breathing rate, while during exercise the cardiac output and muscle blood flow increase and remain high depending on the duration of the exercise and the degree of exertion (Johansen et al., 2003). Work by Johansen et al. (2003) indicated that children with SCD demonstrated impaired cardiac function such as low maximum workload, high blood pressure and heart rate response owing to the less efficient oxygen supply. It has been reported that these subjects have impaired exercise tolerance and may develop ischemia (restriction in blood supply) owing to an imbalance between myocardial oxygen supply and demands (Balfour et al., 1984). A decrease in left ventricular volume in SCD suggested that the left ventricles of these children have difficulty in filling when there is an increasing demand on the myocardium, such as in exercise stress (Balfour et al., 1988; Covitz et al., 1983). Other factors affecting exercise capacity in this population include structural and functional adaptations of the vasculature resulting from chronic anaemia, such as lowered blood flow to tissues owing to abnormal vascular tone and an activated, adhesive endothelium (Kato et al., 2009) and peripheral vascular impairments related to recurrent microvascular occlusion (Callahan et al., 2002).
1.1.10 Chronic inflammation

Chronic inflammation involves the local accumulation of fluid, plasma proteins and leukocytes begun by physical injury, infection or a local immune response. Patients with SCD have chronic inflammation, elevating their WBC (Akohoue et al., 2007). The sickle red cell is an irritant that provokes an inflammatory response, as it obstructs blood flow. Tissues suffer hypo-perfusion and are exposed to inflammatory cytokines, growth factors and other actions of the activated inflammatory cells (Platt, 2000). Patients with SCD are also hypermetabolic and show several abnormalities of the immune system when compared with healthy people (Salman et al., 1996; Singhal et al., 2002). These abnormalities include high leukocyte counts, elevated serum levels of acute phase proteins such as C-reactive protein (CRP) and pro-inflammatory cytokines such as IL-6 (Akohoue et al., 2007; Hibbert et al., 2005). The pro-inflammatory cytokines have been illustrated to be significantly higher in children with SCD. Alterations of inflammatory mediators including IL-6, tumor necrosis factor alpha (TNF-α) and CRP may influence the occurrence of vasco-occlusive episodes in SCD subjects by increasing adhesion of erythrocytes to the endothelium (Pathare et al., 2003). Suggested mechanisms are the activating of the endothelial cells adhesion molecules and facilitating the adhesion receptor interaction with the corresponding ligand on the surface of the blood cell. Another mechanism is by decreasing anticoagulant molecules which increase the RBCs coagulation (Pathare et al., 2003). Hypermetabolism, an abnormal increase in the metabolic rate, is generally associated with cytokine driven inflammation in cases of injury or infection (Cone et al., 1997; Tappy et al., 2000). In children with SCD,
hypermetabolism was strongly related to chronic inflammation demonstrated by elevated levels of CRP and WBC count (Hibbert et al., 2005). Furthermore, Darbari et al. (2006) stated that infections accounted for about 18% of SCD deaths.

Exercise and physical activity induce an increase in plasma concentration of several pro- and anti-inflammatory cytokines, with IL-6 having the most dramatic increase (Ostrowski et al., 1999). In normal healthy subjects, exercise-induced pro-inflammatory cytokines, TNF-α and Interleukins response is balanced by the release of cytokine inhibitors and the anti-inflammatory cytokine IL-10. This balance causes a restriction of the magnitude and duration of the inflammatory response to exercise (Ostrowski, et al., 1999). Pedersen (2006) has reported in a study in healthy subjects that IL-6 is the first cytokine to be released into the circulation in response to exercise and that its concentration increases during exercise. Interleukin-6 has also been reported to remain elevated 24 hours after exercise after maximal and sub-maximal work in healthy subjects (Edwards et al., 2006; Sprenger et al., 1992). The magnitude of the IL-6 response to exercise depends on the exercise intensity, duration, mass of functioning muscle and endurance capacity of the subject (Pedersen, 2006). In adult women with SCD, it has been indicated that exercise does not affect CRP, IL-6, TNF-α and endothelin-1 (ET-1) concentration (Barbeau et al., 2001). However, Barbeau et al., 2001 reported a significant increase in vasoactive NO metabolites, which have a vasodilatory action, and thus it is likely that exercise has beneficial outcomes in these individuals.
To date, no known studies in children with SCD have reported on the effect of exercise in inflammation. The research in adults with SCD suggests a possible positive effect (Barbeau et al., 2001). In normal healthy subjects, research in the inflammatory response to exercise reported conflicting results, although the general idea is that mild to moderate exercise may induce increase in IL-6, TNF-α and CRP concentrations. However, it is still not clear whether exercise may also induce a similar effect in children with SCD.

1.1.11 Lipids and postprandial triacylglyceride in SCD

Dyslipidemia appears to work in a similar mechanism as inflammation in inducing endothelium activation. Lipids are a major source of energy, and they facilitate the absorption of fat-soluble vitamins as well as having key roles in various regulatory functions (Yeum & Russell, 2002). The majority of fat in the diet is in the form of TAG. The remainder consists of cholesterol, phospholipids and free fatty acids (FFA) (Labarthe et al., 2009). The elevated circulating levels of lipids following a meal are known as postprandial lipaemia, and the magnitude of this is considered to be a risk factor for cardiovascular diseases and atherosclerosis (Su et al., 2009). However, data defining the effect of SCD on plasma lipid homeostasis are limited. In SCD patients, decreased oxygen availability has been indicated to limit lipid absorption, transport and endogenous synthesis (Buchowski et al., 2006). Studies addressing SCD have reported elevated levels of TAG plasma concentration in overnight fasting subjects (Muskiet et al., 1984). Elevated serum level of TAG in SCD patients was strongly associated with markers of
haemolysis, endothelial activation and inflammation (Zocra et al., 2009). El Hazmi et al. (1995) indicated a rise in TAG concentrations in responses to painful crisis. Despite lower serum cholesterol concentrations in SCD, LDL from patients with SCD is more prone to oxidation, resulting in the release of free radicals and oxidative stress that contributes to subsequent injury to the endothelium (Belcher et al., 1999).

Exercise bouts performed immediately before intake of high fat meals have been shown to lower postprandial TAG in non-endurance trained people (Herd et al., 2001). Furthermore, several studies have examined the influence of accumulated physical activity one day before consuming a high-fat meal on postprandial TAG concentrations with consistent findings indicating that accumulated physical activity is effective in lowering postprandial TAG concentrations (Barrett et al., 2007; Gill et al., 1998; Tolfrey et al., 2008; Tolfrey et al., 2012). One proposed mechanism is that exercise leads to faster TAG clearance, owing to an increase in the activity of lipoprotein lipase, a major enzyme in the TAG metabolism pathway (Gill & Hardman, 2003). It has been reported that adults who exhibit an increase in the activity of muscle or plasma lipoprotein lipase (LPL) after a single bout of moderate exercise also exhibit a simultaneous reduction in fasting and postprandial TAG concentrations (Gill & Hardman, 2003; Herd et al., 2001). However, the effect of exercise on postprandial TAG in adults and children with SCD has not yet been assessed in the literature.

1.1.12 Growth, Maturation and Performance
Individuals with SCD show a delay in growth and maturation manifested as low weight and height and late skeletal and sexual maturity (Zago et al., 1992). Growth and maturation have been indicated to influence the physical activity and cardiopulmonary fitness in adolescents (Mota et al., 2002). During puberty, endocrine changes result in secondary sexual development, increases in growth velocity and changes in body composition (Loomba-Albrecht & Styne, 2009).

1.1.12.1 Body composition and growth velocity changes in boys

In boys, puberty takes about 2 to 5 years to complete, and the typical sequence of pubertal events is adrenarche, beginning of the growth spurt, genital development, beginning of pubic hair and peak height velocity (Neinstein & Kaufman, 2002). Boys gain significant fat-free and skeletal mass during puberty.

In terms of weight, 40% of adult weight is gained between the ages of 12 to 18 years in males. The weight gain induced by hormonal changes associated with puberty may result in early-maturing males being heavier than age-matched peers (Malina & Bouchard, 1991). A large proportion of growth during adolescence in males is accounted for by the increase in muscle mass. Early-maturing males are found to have significantly greater muscle mass than late-maturing males, although this advanced muscularity might not continue into adulthood (Pearson et al., 2006). In addition, fat increases in boys during childhood, with a marked decrease between ages 14 to 16 years. Fat deposition during puberty is a result of a complex interaction between genetics and hormone activity.
but is also related to behavioural factors, such as habitual activity and nutrition (Pearson et al., 2006).

Peak height velocity is a measure of the maximum rate of growth in stature during a growth spurt. An increase in height during puberty is related to hormonal activity. Therefore, during adolescence, height is strongly related to pubertal stature (Malina & Bouchard, 1991). Early-maturing males are taller than average than late-maturing males during all stages of adolescence (13 to 18 years).

1.1.12.2 Physical activity and fitness

The physiological changes during puberty influence physical activity and cardiopulmonary fitness levels. Mota et al. (2002) showed that sexual maturity status explained a significant amount (5% in boys and 8% in girls) of the variance in fitness level in a given age group. Evidence supporting the influence of maturity status on physical activity has also been established.

**Physiological capacity:** In males, peak oxygen consumption (ml/kg/min) increases steadily between ages 8 and 16 years, with greatest improvement in aerobic capacity found to occur between the ages of 11 and 15 years (Malina & Bouchard, 1991). Increases in aerobic power are related to many factors, such as increases in fat-free mass (FFM), development of the physical and functional size of the cardiovascular system and increased Hb content of the blood (Malina & Bouchard, 1991).
**Anaerobic power:** The highest rate of improvement in anaerobic power occurs between the ages of 14 and 15 years (Falgairette et al., 1991). Anaerobic power production is strongly related to body mass during adolescence; therefore, the effect of body size parameters on anaerobic power performance is strongly linked to maturation status (Pearson et al., 2006).

**Strength:** A steady increase in strength occurs during childhood, followed by a larger improvement during adolescence. The development in strength is related to the development in body size and serum testosterone concentrations that again links strength development to maturation status (Pearson et al., 2006).

1.1.12.3 Growth & Maturation in children with SCD

Studies in patients with SCD showed that they have low mean weight, which is apparent by 2 years of age, and differences increase with age (Zago et al., 1992). There is also a marked delay in the onset of the normal pubertal growth spurt and sexual development (Zago et al., 1992). Zago et al. (1992) studied the growth and sexual maturity of 125 patients with SCD aged 7 months to 42 years, using the Tanner and Whitehouse method. Compared with normal subjects, individuals with SCD showed a delay in sexual maturity and growth. Comparison between adults and young patients indicate that puberty was delayed in SCD patients in both sexes, but normal sexual maturation was attained later in life. Zago et al. (1992) concluded that growth delay in SCD patients was associated with disease syndromes such as tissue hypoxia caused by
low haematocrit (Hct), endocrine dysfunction, the acute effect of vasco-occlusion, chronic organ damage caused by sickling, low dietary intake and low social status.

Leonard et al. (1998) examined skeletal maturation using hand-wrist x-ray and sexual maturation using Tanner stages in 104 children with SCD (< 18 yrs). They concluded that zinc deficiency was strongly associated with degree of growth failure in children with SCD. Leonard et al. (1998) documented that abnormalities of plasma zinc in SCD, depressed level of plasma zinc concentration and erythrocyte zinc deficiency are all associated with growth retardation in SCD children.

Silva and Viana (2002) studied growth deficits in 100 children with SCD aged (< 8 yrs). They stated that the observed delay in growth in SCD children may be associated with fast RBC turnover. Thomas et al. (2000) published reference curves for height and weight in Jamaican SCD children (age range 0 to 18 years). They stated that SCD children follow the same length and weight as normal healthy children at birth and exhibit maximum lag at ages 10 to 15 years. After this, they catch up with growth to be similar to their normal peers. They found that puberty was delayed by an average of two years in SCD children, compared with same-aged normal healthy children in the USA.

Barden et al. (2002) studied body composition in children with SCD aged 5 to 18 years. Growth was assessed by using standard methods of measuring height and weight. Skeletal maturity was assessed using radiography of the left hand and wrist and scored according to the Tanner method. Barden et al. (2002) concluded that SCD children have impaired growth and delayed puberty and that this was associated with poor nutrition.
In summary, the deficit in growth and delayed maturity in SCD are associated with many nutritional, social and physiological factors that contribute to this situation. Although Thomas et al.’s published height and weight reference curves are essential when comparing children with SCD to other children, it is unknown whether these curves are suitable to use in other ethnic groups and geographical origins.

1.1.12.4 Assessing growth and maturation

It is important in children and exercise studies to consider and control for maturity. The indicators most widely used to assess biological maturation in growth studies are skeletal, sexual and somatic maturation (Malina & Bouchard, 1991).

Skeletal maturation is considered to be the best assessment of biological maturity status as it spans the entire period of growth from infancy into young adulthood. The maturation of the skeleton can be assessed using an x-ray of certain portions of the body, particularly the hand and wrist. Other areas of the body can be used, such as the foot, knee and ankle. The hand and wrist are the most common areas used to assess skeletal maturation (Malina et al., 2004). Hand and wrist assessment is based on changes in the developing skeleton that can be observed and evaluated using the x-ray. Several computerised protocols have also been used. The most widely used method is Dual-energy x-ray absorptiometry (DEXA), which measures bone acquisition. DEXA has several advantages, including low cost, minimal radiation exposure, accessibility and simplicity of use (Gilsanz et al., 1997). Major developments in the understanding of genetic and environmental determinants of areal bone mineral density (aBMD) in normal healthy children have been
achieved by the use of DEXA (Gilsanz et al., 1997). Criticism of this technique includes uncertainty and disagreement over the interpretation of DEXA measures as most growth-related increases in DEXA aBMD values are due to increases in bone size rather than bone density (Malina et al., 2004). In addition, gender differences in aBMD values are also neglected as boys usually have greater bone size (Gilsanz et al., 1997).

The second method used in assessing maturity is the measurement of sexual maturation. The assessment of sexual maturity in growth studies is based on the assessment of secondary sex characteristics such as breast and pubic hair development and menarche in girls, and genital and pubic hair development in boys (Malina et al., 2004). Frequently, the criteria used to assess sexual maturity are the stages for pubic hair, breast, and genital maturation described by Tanner and Whitehouse (1962). One limitation to this method is associated with the rating of each stage of sexual maturity, which depends upon the skills of the assessors. The main criticism of the method is its reliance on direct observation, which to some extent involves the invasion of the individual’s privacy. Another criticism of using Tanner’s scale (1962) is that it does not take into consideration the variations among adolescents in reaching each stage of maturity, as some adolescents may exhibit minimal changes during a given stage, followed by a rapid change in characteristics in the next stage. Another method that has been used in studies is the self-assessment method, which also represents the subjective side of the assessment of sexual maturity, although the self-assessment method is reported to be a valid and reliable method to assess sexual maturity in elite adolescent
athletes (Leone & Comtois, 2007) and patients with chronic disease (Boas et al., 1995; Schall et al., 2002).

The third method of assessing maturity is somatic maturity, which focuses on the adolescent spurt in height. Age at peak height velocity (PHV) is the most common indicator of somatic maturity and particularly relates to timing (Bailey, 1997). Peak height velocity (cm/year) provides an estimate of tempo; therefore, if adult size can be estimated, the amount of growth during the adolescent growth spurt, the adolescent gain, can be calculated. However, in such assessment, longitudinal data are required to estimate age at PHV, and with this method the researcher can only estimate maturity during the adolescent period.

Owing to the difficulties surrounding previous methods of assessing maturity, new non-invasive methods have been developed. One of the most applicable non-invasive methods is based on assessment of the present stature relative to predicted adult stature without skeletal age (Roche et al., 1983). The decimal age, height and body weight of the child and height of the parents are used to predict mature (adult) height for the child. The current height of each child is then expressed as a percentage of his predicted mature height in order to provide an estimate of biological maturity status. The percentage of predicted mature height of each child is expressed as a z score so as to classify children into maturity groups. Maturity status is expressed as a z score, using the percentage of predicted adult height at the time of measurement (Cumming et al., 2006). Using this method, children are classified as late (delayed, -1.0), on time (average, -1.0 +
1.0) or early (advanced, greater than +1.0). Malina et al. (2007) stated that this method is reliable, practical and useful, compared with other measurements of maturity in children. The advantages of using this method include its consideration of privacy, avoidance of children being exposed to radiation and ease. The limitations of this method include some possible median errors between actual and predicated mature height (2.2 cm) at 18 years of age in males (Khamis & Roche, 1994). Moreover, this method depends on parents reporting their height who might overestimate the values, leading to inaccurate results (Cumming et al., 2004).

1.1.13 The potential role of regular exercise in SCD

Physical activity has been proved to enhance physical fitness and reduce mortality and morbidity in the general population and to improve quality of life in people with chronic diseases such as metabolic syndrome (Balducci et al., 2009; Colberg & Grieco, 2009) and heart failure (Dubach et al., 2001). It is thus recommended that individuals accumulate 30 minutes of moderate exercise daily. Although SCD is considered a chronic condition, the literature evaluating the safety and effectiveness of exercise in SCD patients is limited (Connes et al., 2011). Physiological changes associated with exercise in healthy individuals include hypoxia, lactic acid accumulation and hyperthermia. These changes explain the concern relating to individuals with SCD performing exercise as they may increase the sickling rate, therefore increasing complications and painful crisis episodes.
The level of physical activity at which patients with SCD may participate in without developing potential complications is still not known (Connes et al., 2011). It has been indicated that the presence of anaemia is responsible for the reduction of oxygen delivery to tissues (Lonsdorfer et al., 1983) and faster transition from aerobic to anaerobic metabolism leading to subsequent accumulation of lactic acid during exercise (Moheeb et al., 2007). These processes may stimulate the polymerization of Hb S (HbS) and increase rate of sickling, thus promoting occlusion of microvasculature. However, some studies have suggested that exercise might be a successful symptom limiting therapy in patients with SCD as different exercise therapy attempts lead to reduction in painful crisis occurrence and reduction of length of hospitalization (Alcorn et al., 1984; Tinti et al., 2010). There is some evidence that moderate intensity exercise might hold positive health outcomes in subjects with SCD, as Barbeau et al. (2001) have demonstrated that exercise at moderate intensity might reduce the inflammatory risk and enhance vasodilatation in adults with SCD. In addition, short incremental cycling exercise induced no haematological or haemorheological changes and resulted in a delayed improvement of the RBC disaggregation, suggesting that exercise might improve the microcirculatory blood flow (Waltz et al., 2012).

The benefits of physical activity during childhood are well established. Children with SCD are known to have several physiological abnormalities, including deficiencies in body size, strength and endurance, and to have cardiopulmonary limitations to exercise that are thought to affect their development of physical performance. Nevertheless, it is important to investigate the beneficial aspects of exercise in these children.
1.1.14 Summary

In summary, the literature indicates that children with SCD have limited exercise tolerance in comparison to normal healthy children. Several factors contribute to their exercise intolerance, including pulmonary, cardiac and immune system abnormalities that are related to their abnormal Hb and its subsequent physiological manifestations. As SCD is considered to be an inflammatory condition, and given the effect of exercise in the inflammatory markers, it is expected that exercise will influence the health of these individuals. Inflammatory cytokines such as IL-6, TNF-α and CRP and postprandial TAG have been reported to have direct effects on inflammation in SCD. Exercise has been shown to have positive health effects in healthy children, while others reported possible positive and negative outcomes in adults with SCD. However, studies investigating the effect of exercise in SCD children are limited. Despite the proposed negative effects, it is important to investigate the possible positive health outcomes of exercise on these children. Therefore, the objectives of the following work are:

- To determine physical fitness and body composition characteristics in Omani children with SCD and SCT compared with normal healthy children.
- To determine whether an acute bout of exercise elicits potentially beneficial effects in Omani children with SCD.
2. MATERIALS AND METHODS

2.1. Introduction

The experimental procedures described in Chapter 3 took place in the laboratories of the Department of Physical Education at Sultan Qaboos University in Oman. The experimental procedures described in Chapters 4 and 5 took place at the Cardiac Unit of Sur Regional Hospital in Oman. The study described in Chapter 6 was conducted at Sultan Qaboos University Hospital. Each study received ethics approval from the local hospital ethics committee.

All participants in the studies included in this thesis were male children from Oman aged between 12 and 15 years. The participants and their parents were given a detailed information sheet that described the aims, procedures, possible risks and requirements of each study. Those who agreed to participate in the experiments completed a written informed consent form and were asked to complete the Leisure-Time Exercise Questionnaire (Godin & Shephard, 1985). Participants with a history of blood transfusion, stroke, hypertension, vaso-occlusion or serious cardiac arrhythmias were excluded from all studies. A staff nurse and a physician were present for all trials in the experimental studies described in Chapters 4, 5 and 6.

2.2. Indices of flexibility, strength and power

Four measurements were taken: vertical jump, back strength, grip strength and flexibility (Dougherty et al., 2011; Moheeb et al., 2007). All tests were performed after
three minutes of warm-up. The instrument used for each measurement was calibrated prior to the start of data collection to ensure that each measurement was accurate and reliable. The dominant side of each participant was determined by observing which hand the participant uses to write his name in an attendance form in the first day of the trials. The purpose of the test was explained for each participant prior to performing the test.

Vertical jump was measured using a digital display (Takei Scientific Instrument Co., Japan). Children were instructed to smoothly flex their knees to 90°, and jump as high as they could. To complete the jump, they fully extended their knees and swung their hands while jumping. To familiarise the children with the test procedures, they were all instructed to practice two jumps before performing the actual test trials. Two test jumps were then performed, and the jump with the better score was recorded in centimetres.

A back strength dynamometer (Takei Scientific Instrument Co., Japan) was used for the measurement of back and leg strength. For the leg strength measurement, children were instructed to stand with the scapulae and buttocks positioned flat against a wall, and knees slightly flexed. The pull bar of the dynamometer was then placed in the hands, with palms facing toward the body. The chain length was adjusted so that both arms were extended. Keeping their arms straight, and without bending their back, children were instructed to pull the bar as hard as possible in a smooth and steady manner. Children
practiced two trials before performing the actual test trials. Two test trials were then performed, and the one with the better score was recorded in kg.

For the measurement of the back strength, children were instructed to stand on the foot-plate of the Takei back and leg dynamometer with their knees fully extended and feet at shoulder-width distance. While maintaining an upright posture with arms and legs straight, they were instructed to place the palm of the hands in front of the thighs. Chain length was adjusted at fingertip level. Children were instructed to lean slightly forward at the hips with head and trunk erected. They grasped the hand bar with one palm facing forward and the other backward. They were instructed to pull the hand bar straight upward using the back muscles and to roll the shoulders backward during the pull, without leaning backward. Children practiced two trials before performing the actual test trials. Two test trials were then performed, and the one with the better score was recorded in kg.

A grip strength dynamometer (Takei Scientific Instrument Co., Japan) was used for the measurement of grip strength using the right hand (dominant side for all participants). The handle of the dynamometer was adjusted to the child’s hand size. The children were seated on a chair with the shoulder adducted. The elbow was flexed at 90º and the forearm was held in a neutral position. After detailed instructions, the children were encouraged to give their maximum effort squeezing onto the dynamometer. The duration of the contraction was 3 to 5 seconds. Children practiced two trials before
performing the actual test trials. Two test trials were then performed, and the one with the better score was recorded in kg.

Flexibility was measured using a sit-and-reach box (Acuflex, USA). Children were instructed to sit on the floor with their back straight against a wall and legs stretched out straight ahead. They were instructed to remove their shoes. The soles of the feet were placed flat against the box. Both knees were locked and pressed flat to the floor. With palms facing downwards, and hands side by side, ensuring that both hands were at the same level, children were instructed to reach forward along the measuring line as far as possible. Children were instructed to hold that position for one-two seconds while the distance was recorded. Readings were taken at the farthest point reached along the scale. Children practiced two trials before performing the actual test trials. Two test trials were then performed, and the one with the better score was recorded in centimetres.

2.3. Anthropometry

Height was measured to the nearest 0.01 cm using a fixed digital stadiometer (Holtain Ltd, UK). Weight was measured to the nearest 0.05 kg using a digital electronic clinical scale (Weylux, UK) with subjects wearing light clothing and no shoes. Body Mass Index (kg/m$^2$) was calculated for all participants. In the study described in Chapter 3, skinfold thickness was recorded at four sites (triceps, biceps, sub-scapula and supra-illiac), and the Durnin and Womersley (1974) equation for skinfold thickness was applied. In the studies described in Chapters 4 and 5 skinfolds were measured at two sites (sub-scapula and triceps) using Harpenden Skinfold Callipers (John Bull, England).
Percentage body fat was calculated using the sex-specific Slaughter equation for boys (Slaughter et al., 1988). The equation for boys is as follows: percentage fat = 1.21 (triceps + subscapular) – 0.008(triceps + subscapular)² – 3.2. All measurements were taken from the right-hand side of the body with the child standing in the anatomical position. In the study described in Chapter 6, body surface area (BSA) was derived using Haycock equation BSA = 0.024265 X W^{0.5378} X H^{0.3964} which was validated for infants, children and adults (Haycock et al., 1978).

2.4. Physical Activity Questionnaire

In order to assess physical activity, participants were asked to complete the Leisure-Time Exercise Questionnaire (LTEQ: Godin & Shephard, 1985). The LTEQ is a two-item questionnaire (with numerous sub-questions assessing specific kinds of physical activity) about usual leisure-time exercise habits. Participants are asked to consider the past seven-day period and report how many times they engaged in specific kinds of exercise for more than 15 minutes during their free time. Exercise activities are classified as strenuous (e.g., heart beats rapidly - running), moderate (e.g., not exhausting - fast walking), or mild (e.g., minimal effort - fishing) exercise. Weekly frequencies are multiplied by 9, 5, and 3 for strenuous, moderate, and mild exercise, respectively. A composite score is calculated by summing the products of each component. The second item in the questionnaire asks participants how many times over the past week they engaged in physical activity long enough to work up a sweat. This questionnaire has
demonstrated adequate reliability and validity in adults (Jacobs, Ainsworth, Hartman & Leon, 1993).

2.5. Maturation

Maturation in children with SCD, SCT and normal children was assessed using a non-invasive method using parents’ height (Khamis & Roche, 1994) either when the parents were at the hospital or by contacting them later in the same week. The decimal age, height and body weight of the child and height of the parents were used to predict mature (adult) height for the child. The current height of each child was then expressed as a percentage of his predicted mature height in order to provide an estimate of biological maturity status. The percentage of predicted mature height of each child was expressed as a z score so as to classify children into maturity groups. The maturity status of each child was classified on the basis of his z score for percentage of mature height (Malina et al., 2005). A z score between -1.0 and + 1.0 was taken to represent that a child’s maturation was average or ‘on time’; a z score below -1.0 was taken to represent late or delayed maturation; a z score greater than + 1.0 was taken to represent early or advanced maturation (Malina et al., 2005).

2.6. Multi-Stage Fitness Test

The multi-stage fitness test (MFT) (Leger & Lambert, 1982) consists of shuttle running between two parallel lines set 20 m apart. Running speed is indicated by signals emitted from a pre-recorded audiocassette tape. The audiocassette tape dictated that participants started running at an initial speed of 2.36 m.s\(^{-1}\) and that running speed
increased by 0.14 m.s\(^{-1}\) each minute. All participants performed a 10-minute warm-up that included prescribed jogging and stretching. The MFT was conducted in a gymnasium at the Sur sport complex with sprung wooden flooring, where participants ran in groups of five in order to add an element of competition and to aid maximal effort. All participants were verbally encouraged to perform maximally during each assessment. The test was stopped if the participant failed to reach the line (within 2 m) for two consecutive shuttles. After finishing the MFT, all subjects participated in a five-minute cool-down that also included prescribed jogging and stretching. The MFT results for each participant were expressed as a predicted VO\(_2\)max (ml kg\(^{-1}\) min\(^{-1}\)) obtained by cross-referencing the final level and shuttle number (completed) at which the participant stopped with that of the VO\(_2\)max table provided in the instruction booklet accompanying the MFT. Participants were familiarised with the test before completing it on the day of the trial. All subjects were fully familiarised with the test protocol before data collection.

2.7. **Treadmill Exercise Test**

In the study described in Chapter 3, in the absence of exercise guidelines for this population, a 5-minutes treadmill walking test was used as a stress test. This test has been considered to be safe and was used for people with chronic conditions (Price *et al.*, 1988). In studies described in Chapters 4 and 5, a modified Bruce protocol was used to assess aerobic fitness. This protocol has been widely used with high-risk and elderly individuals (Heyward, 2002) and has been used in individuals with SCD (Mani *et al.*, 2005). The test consists of seven steady state exercise stages; each lasting three minutes (see Table 2.1).
Participants walked on the treadmill until exhaustion or until reaching their maximal heart rate of 175 bpm for children with SCD, as advised by Mani et al. (2005). Time was recorded from the first minute of the test until the participant was unable to continue. None of the participants completed the seven exercise stages in both studies. Heart rate and blood pressure were measured at the end of each 3-minute stage and also every three minutes during recovery for 15 minutes after exercise. The CardioSys-series 2000 treadmill with a Cardamax-fx 2111-ECG (USA) was used for this test. Calibration of the treadmill speed was carried out according to the manufacturer’s instructions.

Table 2.1: Modified Bruce Protocol

<table>
<thead>
<tr>
<th>Stage</th>
<th>Duration (min)</th>
<th>Total time</th>
<th>Speed (km/h)</th>
<th>Grade (%)</th>
</tr>
</thead>
<tbody>
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<td>2.7</td>
<td>0</td>
</tr>
<tr>
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<td>3</td>
<td>6</td>
<td>2.7</td>
<td>5</td>
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<td>2.7</td>
<td>10</td>
</tr>
<tr>
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<td>12</td>
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<td>12</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
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<td>14</td>
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<td>7</td>
<td>3</td>
<td>21</td>
<td>8.0</td>
<td>18</td>
</tr>
</tbody>
</table>

2.8. Blood sampling and heart rate measurements

All participants reported to the laboratory following an overnight fast. In all studies, a trained staff nurse was present to take blood samples. Haemoglobin, Hct, WBC
count and RBC count were measured using an automatic haematology system (SF-3000, Sysmex, Milton Keynes, UK). An Accutrend Portable Lactate Analyser (Roche Diagnostics GmbH, Germany) was used for the analysis of blood lactate concentrations. Heart rate was measured by short-range telemetry (Polar, PE 3000, Kempele, Finland).

In Chapter 5, venous blood samples were taken from an anterior cubital vein. A maximum of 5 ml was drawn for each sample into an EDTA tube. The samples then were kept at 4°C until centrifugation. Plasma was separated and stored at – 40°C until it was transported to Bath for the analysis of IL-6. A commercially available enzyme-linked immunosorbent assay (ELISA) kit was used to analyse IL-6 (Quantikine, R & D Systems, Abingdon, UK).

In the study described in Chapter 6, capillary blood samples collected into EDTA tubes during the oral fat tolerance test were analysed for total cholesterol, HDL cholesterol, LDL cholesterol, glucose concentration TAG and insulin (fasting samples). Glucose levels were measured using enzymatic methods (Cobas Integra 800; Roche, Switzerland). The levels of other biochemical markers; serum TAG, total cholesterol, HDL and LDL were also measured by enzymatic methods (Cobas Integra 800; Roche, Switzerland). Serum insulin was determined using standard fluoroimmunoassays (AutoDELFIA, Finland). Serum CRP was measured using competitive immunoassay (Roche Diagnostics, Germany).
3. PHYSICAL FITNESS IN OMANI CHILDREN WITH
SICKLE CELL DISEASE AND SICKLE CELL TRAIT

3.1. Introduction

Sickle cell disease and SCT are the most common haematological diseases in Oman, according to the national survey of genetic blood disorders (Al-Riyami & Ebrahim, 2003). Sickle cell disease is caused by a genetic mutation that causes polymerisation of RBCs during Hb de-oxygenation, resulting in a characteristic rigid sickle shaped RBC (Kaul et al., 1989). The rigid sickle cell can obstruct blood flow in small blood vessels, leading to painful crisis, organ damage (Goodman, 2004) and stroke (Serjeant, 1997). Sickle cell trait is characterized by the presence of both normal Hb (HbAA) and sickle Hb (HbSS). Usually, SCT causes fewer complications in carriers, since they have an adequate quantity of HbAA in the RBC to function normally.

Several studies have reported differences in growth patterns, body composition and physical fitness in adolescents and children with SCD, compared to normal healthy. The differences include delayed puberty, skeletal immaturity and deficit of weight and height (Luban et al., 1982; Phebus et al., 1984; Platt et al., 1984). These growth manifestations seem to be more marked in male patients (Reed et al., 1987). Studies designed to estimate body composition in children with SCD indicated that these children are shorter and lighter and have a greater proportion of their body mass as fat (Barden et al., 2002; Moheeb et al., 2007; Zemel et al., 2007). Thomas et al. (2000) stated that
children with SCD might catch up with normal healthy children in achieving normal height in early adulthood but that their weight typically remains below average. In addition, Dougherty et al. (2011) indicated attenuated maximal muscle strength and peak power in 5- to 13-year-old African-American children with SCD. The lower body mass in children with SCD appears to explain their lower performance in strength measures (Dougherty et al., 2011).

Traditionally, physicians’ recommendations for individuals with SCD and SCT have been to avoid physical activity because it may increase the rate of haemolysis (premature destruction of erythrocytes, which leads to haemolytic anaemia when bone marrow activity cannot compensate for the erythrocyte loss) and aggravate the complications of the disease (Connes et al., 2008). Martineaud et al. (2002), in an exercise performance study in young subjects with SCD, stated that physical exertion may lead to physiological changes that enhance polymerization of the abnormal HbSS. Physical activity may increase the rate of Hb de-oxygenation, which in turn increases the rate of sickling and haemolysis. Haemolysis of RBC increases the chance of RBCs adhering to the vascular endothelium (Campbell et al., 2009), which may result in painful crisis due to vascular occlusion (Davis, 1988; Eichner, 1993; Francis & Bleakley, 1980; Scheinin & Wetli, 2009). The clinical manifestations resulting from tissue hypoxia and vascular injury depend on the concentration of HbSS and the severity of sickling and present as lower exercise tolerance (Martineaud et al., 2002).
In recent years, many studies have provided support for the hypothesis that regular physical activity has health benefits in the general population (e.g., Altena et al., 2004; Schmidt et al., 2001). The Centre for Disease Control and Prevention and the American College of Sports Medicine recommend that adults aged 18 to 65 years accumulate at least 30 minutes of moderate-intensity aerobic activity five days per week or engage in 20 minutes of vigorous activity three days per week (Haskell et al., 2007). More specific guidelines for children have been developed by the World Health Organization (WHO), recommending that children and young people aged 5 to 17 years accumulate at least 60 minutes of moderate- to vigorous-intensity physical activity every day (WHO, 2010). While the focus of the guidelines is that most of the daily physical activity should be aerobic, it is suggested that vigorous-intensity activities be incorporated, including those that strengthen muscle and bone, at least three times per week. Although there are no exercise guidelines for SCT/SCD individuals, some studies have suggested beneficial effects for exercise in these populations. Aufradet et al. (2010) studied the effect of habitual physical activity on endothelial activation in adults with SCT. The results of the study concluded that a physically-active lifestyle may have a beneficial impact, as it may decrease endothelial activation and limit the risk of vascular adhesion events in the microcirculation in SCT population. Furthermore, Chirico et al. (2012) investigated the role of exercise training on several biomarkers of oxidative stress in adults with SCT. Their data indicated that the overall oxidative stress and NO response is improved in trained, compared to untrained, SCT individuals, suggesting that physical activity might have a beneficial effect on oxidative stress and the associated vascular
impairment in this population. Waltz et al. (2012) investigated the beneficial effect of short incremental cycling exercise tests in patients with SCD. The study concluded that exercise resulted in an improvement of the RBC disaggregation in the SCD group, suggesting that exercise might improve the microcirculatory blood flow. Although the investigations into the effect of exercise in SCD subjects have reported beneficial outcomes, further investigation into physical fitness in this population is still needed. The aim of this study was to assess a range of markers of physical fitness in children with SCD and children with SCT and compare them with normal healthy children of the same age.

**Hypothesis**

Due to the presence of Hb S and its effect on the health and lifestyle of its carriers, and given the influence of exercise on the physiological characteristics of Hb, it was expected that subjects with Hb S will show reduced physical fitness parameters compared with normal healthy subjects with Hb A, and those carrying the homozygous form of Hb S (SS) will have poorer physical fitness than those carrying the heterozygous form (AS).

**3.2. Methods**

**Subjects**

One hundred and twenty male children aged 12 to 15 years participated in this study (see Table 3.1). The children belonged to one of three groups: 40 children with
SCD; 40 children with SCT; and 40 normal healthy children with normal Hb. The SCD and SCT children were recruited through the Paediatric Haematology Department at Sultan Qaboos University Hospital (SQUH) in Oman. Normal healthy children were recruited from local schools in the capital city. Children with a history of stroke, serious cardiac arrhythmia, vaso-occlusion, hypertension or blood transfusion or those taking any medication were excluded from this study. The study protocol was approved by the Medical and Ethical Research Committee at SQUH. Data were collected in the Physiology Laboratory, Department of Physical Education, College of Education, Sultan Qaboos University. Body mass, body height and skinfold thickness were measured as described in Chapter 2.

**Experimental Design and procedures**

*Treadmill exercise test*

In the absence of exercise guidelines for this population; a five-minute walking test on a treadmill was performed by all participants at a speed corresponding to 5 km/h without any incline. This test was performed as a stress test in order to determine the heart rate and blood lactate responses to exercise (Price et al., 1988). The test was terminated if a subject exhibited symptoms of inability to continue, such as ECG abnormalities, heart rate above 175 bpm or signs of exhaustion. (A cardiologist was present for all trials).
Resting venous blood samples were taken after participants had rested for ten minutes in a sitting position, for the measurement of Hb levels. Further samples were taken immediately after exercise for the analysis of blood lactate concentrations (Accutrend Portable Lactate Analyzer, Roche Diagnostics GmbH, Germany). Heart rate was measured at rest, during exercise and 10-minutes after exercise by short-range telemetry (Polar, 3000, Kempele, Finland) (see Figure 3.1).

Figure 3.1: Experimental protocol

Key: ♥ → Blood samples, ▼ → Heart rate, ♦ → Resting Hb

♥ venous blood samples for main trial. Black arrow shows exercise time, dashed arrows shows recovery time.
Indices of strength, power and flexibility

Following a short familiarisation period, children performed three strength tests; leg, back and hand-grip strength. A back strength dynamometer (Takei Scientific Instrument Co., Japan) was used for the measurement of back and leg strength. A grip strength dynamometer (Takei Scientific Instrument Co., Japan) was used for the measurement of hand-grip strength. The dynamometer was adjusted for each subject’s hand size. Vertical jump was used to determine the explosive power of the leg muscles (Takei Scientific Instrument Co., Japan). Flexibility was measured using a sit-and-reach test (Acuflex, USA). In all strength, power and flexibility tests, children were strongly encouraged to give a maximum effort (see Chapter 2.2).

Statistical analysis

One–way analysis of variance (ANOVA) was used to investigate differences among the three groups for the following dependent variables: age, weight, height, vertical jump, leg strength, back strength, grip strength (right hand), flexibility and resting Hb. Two-way ANOVA with repeated measures was used to detect differences in mean values among the groups for heart rate and blood lactate. Where appropriate, Tukey’s post-hoc tests were employed to ascertain which mean values were significantly different. A Pearson correlation was used to investigate relationships between resting Hb levels and peak heart rate as well as FFM and strength measures in all groups combined.
All data are presented as mean (SD). Data were analysed using SPSS version 17 (SPSS Inc., Chicago, USA). Significance was accepted at $P < 0.05$.

### 3.3 Results

#### Anthropometric measure

ANOVA revealed a significant difference in age in SCD children compared with normal healthy and SCT children. Body mass and body height were significantly different among groups, with greater ($P < 0.05$) body mass and height in children with SCT and in the normal healthy group than in children with SCD (see Table 3.1). Body fat percentage and body mass index (BMI) were significantly different ($P < 0.05$) among groups. The SCD group had a significantly higher body fat percentage ($P < 0.05$) than the SCT and control groups, but a significantly lower BMI ($P < 0.05$).

Table 3.1: Mean (SD) age and anthropometric measures in the three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Control group (n=40)</th>
<th>Sickle cell trait (n=40)</th>
<th>Sickle cell disease (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>12.8(.8)</td>
<td>12.8(.9)</td>
<td>13.3(.9)*</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>37.2(4.3)</td>
<td>37.9(4.3)</td>
<td>28.8(3.0)*</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>139.4(8.7)</td>
<td>141.3(9.9)</td>
<td>132.5(3.2)*</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>17.5(6.1)</td>
<td>17.0(4.2)</td>
<td>12.9(3.0)*</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>31.9 (7.1)</td>
<td>31.8(3.6)</td>
<td>36.1(8.1)*</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>25.5(4.0)</td>
<td>25.0(3.4)</td>
<td>19.0(3.9)*</td>
</tr>
</tbody>
</table>

*Significantly different from SCT and healthy controls, ($P < 0.05$).

#### Flexibility, strength and power
The SCT and control groups had significantly higher ($P < 0.05$) grip strength of the dominant hand, back strength, leg strength, vertical jump and flexibility, compared with the SCD group (see Table 3.2). Pearson correlation indicated a weak positive correlation between FFM and leg strength ($r= 0.18, P < 0.05$), indicating that greater the FFM was associated with a greater leg strength. There was also a weak positive correlation between FFM and grip ($r= 0.26, P < 0.05$) and FFM and back strength ($r= 0.19, P < 0.05$), indicating that greater FFM was also associated with a greater grip and back strength. In addition, Pearson correlation indicated a medium-positive correlation between FFM and vertical jump ($r=0.43, P < 0.05$).

Table 3.2: Mean (SD) flexibility, strength and power in the three groups

<table>
<thead>
<tr>
<th></th>
<th>Control group (n=40)</th>
<th>Sickle cell trait (n=40)</th>
<th>Sickle cell disease (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertical jump (cm)</td>
<td>23.5(5.8)</td>
<td>23.9(4.1)</td>
<td>17.1(4.6)*</td>
</tr>
<tr>
<td>Leg strength (kg)</td>
<td>28.3(9.9)</td>
<td>32.1(10.5)</td>
<td>22.9(4.2)*</td>
</tr>
<tr>
<td>Back strength (kg)</td>
<td>31.6(10.2)</td>
<td>30.8(7.4)</td>
<td>23.1(4.5)*</td>
</tr>
<tr>
<td>Grip strength (kg)</td>
<td>15.5(5.2)</td>
<td>14.8(3.2)</td>
<td>12.4(3.4)*</td>
</tr>
<tr>
<td>Flexibility (cm)</td>
<td>-0.3(4.8)</td>
<td>-2.2(5.5)</td>
<td>-2.8(6.2)</td>
</tr>
</tbody>
</table>

*Significantly different from SCT and control ($P < 0.05$).

**Heart Rate**

All subjects completed the exercise bout. Both the normal and the SCD children recorded a peak heart rate at minute 5 of the exercise. Mean heart rate in the SCD (173 (10.7) bpm) was close to the highest recommended heart rate (175 bpm) for this...
population, while the normal healthy children recorded a peak heart rate of 147 (22) bpm. On the other hand, SCT children recorded a peak heart rate of 152 (14.4) at minute 4 of exercise. ANOVA revealed a significant main effect of time ($P < 0.005$) with a large effect size (eta squared= .978). There was a significant interaction among the groups ($P <0.005$). The *post hoc* Tukey procedure showed that SCD children demonstrated a higher heart rate response during exercise ($P < 0.005$). Heart rate during exercise was higher in the SCD group, compared with the normal healthy children. The main effect of the group was significant ($P < 0.05$) with a small effect size (eta squared= .112).

![Figure 3.2 Mean (SD) heart rate for normal healthy, SCT and SCD children.](image)

*Significantly higher during exercise than SCT and normal healthy ($P < 0.05$).
**Blood parameters**

*Haemoglobin*

There was a significant difference ($P < 0.05$) in resting blood Hb concentration among the three groups. Resting mean values of Hb were similar in the SCT and normal healthy children, while the SCD group showed significantly lower ($P < 0.05$) mean values (Figure 3.3). Pearson correlation indicated a strong-negative correlation between resting Hb levels and peak heart rate when the data for the three groups were combined ($r = -.515, P < 0.05$).
Figure 3.3: Mean (SD) Hb concentration at rest for normal healthy, SCT and SCD children. *Significantly lower in SCD than SCT and normal healthy children ($P < 0.05$).

**Blood lactate**

![Blood lactate graph]

Figure 3.4: Mean (SD) changes in blood lactate for normal healthy, SCT and SCD children at rest and after exercise. *Significantly higher blood lactate concentrations in SCD than SCT and normal healthy ($P < 0.05$).

ANOVA revealed a significant main effect of time in blood lactate ($P < 0.005$) with a medium effect size (eta squared= .661). There was a significant interaction among groups over time ($P < 0.05$). The main effect of group was significant ($P < 0.05$) with a medium effect size (eta squared= .445). Post-hoc tests revealed that the SCD group had higher blood lactate at rest and after exercise ($P < 0.05$) than both SCT and normal healthy children.
3.4. Discussion

Currently, limited data are available describing exercise performance in children with SCD or SCT. The main aim of this study was to assess physical fitness parameters and responses to sub-maximal exercise in male children with SCD and compare them to the parameters of children with SCT and normal healthy children. The results of this study indicated that, despite being slightly older than the children in the other groups, children with SCD were shorter and lighter and had a greater proportion of their mass as fat. In addition, they were weaker and less powerful and had exaggerated heart rate and lactate responses to five minutes of treadmill walking.

Several studies report differences in growth patterns in adolescents and children with SCD compared to normal healthy controls. The differences include delayed puberty, weight and height deficits, and skeletal immaturity (Luban et al., 1982; Phebus et al., 1984; Platt et al., 1984). These growth manifestations seem to be more marked in male patients (Reed et al., 1987). The results of the present study indicated that the mean values of body mass and body height were significantly lower in children with SCD, compared with normal healthy children and children with SCT ($p < 0.05$). These results were in agreement with similar studies designed to estimate body composition in children with SCD (Barden et al., 2002; Moheeb et al., 2007; Zemel et al., 2007). The possible reason for the smaller stature and mass might be the poor growth and poor nutritional status of SCD children (Silva & Viana, 2002) or might be due to a deficiency in growth hormone (GH), as observed by Nunlee-Bland et al. (2004). Growth hormone has been
indicated to have a direct effect on growth and body composition in children, and GH deficiency is related to impaired weight, BMI and bone development (Hogler et al., 2005).

Barden et al. (2002) assessed the growth, nutritional status and body composition of 36 children aged 5 to 18 years diagnosed with SCD. Children in the control group weighed significantly more (41.6 ± 15.5 kg) than the SCD group (33.9 ± 13.3 kg) ($P=0.038$), but no data for children with SCT were reported. Thomas et al. (2000) stated that children with SCT might catch up with normal healthy children in achieving normal height in early adulthood, but that their weight typically remains below average.

In the present study, body fat percentage was significantly higher in SCD, compared with SCT and normal children, and it is possible that a lack of regular exercise and unhealthy nutritional habits may have contributed to these findings in children with SCD. Moheeb et al. (2007) reported similar results to the present study in children aged 9 to 12 years. Other studies carried out in adults have reported similar findings of obesity with high levels of body fat and body fat percentage (Pells et al., 2005; Woods et al., 2001). In the present study, the lower FFM reported by SCD children (suggesting poorer bone and muscle development) might explain their lower BMI. These findings concluded that although children with SCD were shorter and had a higher body fat percentage, they weighed less than the SCT and the normal healthy children, which might be related to the endocrine dysfunction in this population reported by Nunlee-Bland et al. (2004).
In the present study, all strength scores were lower in the SCD children than the SCT and the normal healthy children. These findings likely relate to the deficit in body mass and body height and the greater body fat percentage described above. The analysis of the correlations between the FFM and the strength and power scores indicated that the lower FFM of SCD children is associated with lower scores for this population, compared to SCT and normal healthy children. Armstrong and Van Mechelen (2000) stated that muscle development is a key aspect of normal growth and development in boys and that strength increases in proportion to body size. In the present study, the lower body mass in children with SCD appears to explain their lower performance in strength measures. These findings support those reported by Dougherty et al. (2011) in a recent study examining the maximal muscle strength and peak power in 5- to 13-year-old African-American children with SCD. The results showed attenuated maximal muscle strength and peak power in children with SCD, compared with normal healthy children.

Heart rate at rest was similar in all study groups, but children with SCD had higher mean heart rates during exercise compared with children with SCT and normal healthy children. Weisman et al. (1988) reported that cardiopulmonary and gas exchange responses to a brief period of strenuous exercise were comparable with normal values in individuals with SCT. Peak oxygen uptake and heart rate values have also been reported to be similar in individuals with SCT and healthy controls during an incremental exercise test (Sara et al., 2003). The greater increase in heart rate in SCD children may be related to their chronic state of hypoxemia. The lower Hb concentration in children with SCD suggests that they have a lower oxygen-carrying capacity. This is likely to influence
oxygen delivery to working muscles during exercise, and therefore a higher heart rate is required to deliver sufficient oxygen. Indeed, there was a strong correlation between resting Hb concentrations and peak heart rate (r = -0.472, P < 0.05), indicating that low levels of Hb are associated with higher peak heart rates.

The results of the present study showed that children with SCD had a higher baseline blood lactate concentration, compared to the SCT and the normal healthy children. These findings supported those of Petto et al. (2010), who reported that the baseline blood lactate in SCD and SCT individuals were 3 to 4 times higher than the normal healthy individuals. Petto et al. (2010) explained these findings by increased production of lactate, reflecting the transition from aerobic to anaerobic metabolism, or by a reduction in the lactate buffering capacity of the body. As the sickling process leads to fewer functional RBCs, causing a reduced oxygen supply to body tissues, less oxygen is available for the body to convert lactate into other reusable compounds, leading to its accumulation and release into the bloodstream (Petto et al., 2010). However, resting blood lactate was higher than expected, which could be a result of the blood-collection method or of the children not being properly rested. In the present study, SCD children exhibited higher blood lactate in response to exercise (O₂ demand exceeds O₂ supply), indicating a predominant anaerobic metabolism as the major energy pathway. Explaining the physiological role of lactate during exercise in children, Rowland (2005) stated that during low-intensity exercise, pyruvate is the main end product of glucose breakdown and is metabolised by pyruvate dehydrogenase (PDH). Osuagwu (2009) stated that the higher lactate concentration in SCD is explained by the apparent lack of PDH complex
and that lactate accumulation is secondary to pyruvate accumulation. The results of the present study showed that children with SCD a higher lactate response to exercise than both normal healthy and SCT children. However, the present study did not investigate the role of PDH. This study is one of the very few studies conducted to measure the physical fitness of children with SCD (Dougherty et al., 2011; Millis et al., 1994). Millis et al. (1994) studied the effect of routine physical activities (swimming and potato-foot race) in SCD children; the results showed that children with SCD had poor performance in long-distance competitions, compared with normal healthy children.

In summary, the results of this study suggest that children with SCT demonstrate similar anthropometric measurements, physical fitness and exercise responses to normal healthy children. In contrast, SCD children have lower anthropometric measures; lower body mass, higher fat mass and lower physical fitness than SCT and normal healthy children. In addition, in response to exercise, children with SCD exhibit higher heart rate and blood lactate responses than SCT children and normal healthy children. The measurement of the anthropometric patterns in this population will serve as a useful reference for further future studies related to physical health science in this population. The absence of anthropometric measurements of the parents is a limiting factor in this study as it is documented that the height of the parents is an important factor in determining a child’s height and that the physical and nutritional status of the parents may play a central role in the growth and development of the child. Assessing the physical activity level and maturation status of the participants would also be helpful in future studies.
4. CARDIOVASCULAR RESPONSES TO EXERCISE IN OMANI CHILDREN WITH SICKLE CELL DISEASE OR SICKLE CELL TRAIT, COMPARED WITH HEALTHY CONTROLS

4.1. Introduction

Sickle cell disease is a genetic disease characterized by the production of abnormal Hb. An association linking the presence of SCD and SCT in adults with limited exercise capacity has been established (LeMura & Von Duvillard, 2004). Although limited data are available, it is likely that SCD/SCT will affect exercise capacity and exercise responses in children as well. The results presented in the previous chapter suggested that children with SCT exhibit similar physical fitness and exercise responses to normal healthy children. On the other hand, SCD children have smaller stature, lower body mass, higher fat mass and lower physical fitness, compared to SCT children and normal healthy children. The SCD exhibited poorer performance in tests of grip strength, back strength, leg strength, vertical jump and flexibility than both normal and SCT subjects. In addition, children with SCD exhibited higher heart rate and blood lactate in response to exercise than children with SCT and normal healthy children.

A number of factors may contribute to limiting exercise capacity in SCD/SCT patients. The main factor is likely to be reduced oxygen-carrying capacity due to low Hb concentrations (Anthi et al., 2007), given that oxygen delivery is a limiting factor for
VO₂\text{max}. Previous research in adults with SCD showed that these subjects exhibit lower VO₂\text{max}, lower anaerobic threshold, higher heart rate and higher ventilation in response to exercise, indicative of the involvement of several mechanisms in limiting the exercise capacity of this population (Callahan et al., 2002). Other factors affecting exercise capacity in this population include structural and functional adaptations of the vasculature resulting from chronic anaemia, such as abnormal vascular tone and an activated, adhesive endothelium that results in lowered blood flow to tissues (Kato et al., 2009); peripheral vascular impairments related to recurrent micro-vascular occlusion (Callahan et al., 2002); and pulmonary changes resulting from repeated episodes of ACS (Platt 2000). Micro-vascular embolism, pulmonary hypertension, increased airway reactivity, interstitial lung abnormalities such as endothelial dysfunction, and parenchymal fibrosis are suggested mechanisms of pulmonary changes contributing to limiting exercise capacity (Maire et al., 2000; Siddiqui & Ahmed, 2003).

Exercise testing is commonly used as a physiological stress or to identify cardiovascular abnormalities. Many studies have used exercise testing to measure physiological stress in the cardiovascular system and abnormalities of cardiac function in children with SCD (Braden et al., 1996; Delclaux et al., 2005; Oyono-Enguelle et al., 2000). The aim of this study was to assess the cardiopulmonary response of SCD/SCT and normal healthy children from Oman using the multi-stage fitness test and a sub-maximal exercise test. The primary measure of this study was the estimation of VO₂\text{max} using the multi-stage fitness test as a measure of cardio-respiratory fitness (Bassett &
Howley, 2000). The secondary measures of this study were heart rate and blood pressure, before, during and after sub-maximal exercise.

Hypothesis

Subjects with SCD were expected to have lower cardiopulmonary fitness. It was expected that VO$_2$ max, as assessed by the multi-stage fitness test, would be lower in children with SCD and that blood pressure and heart rate responses to sub-maximal exercise would be exacerbated.

4.2. Methods

Subjects

The participants were 27 Omani male children aged 12 to 15 years. Eight of the children had been diagnosed with SCD, 9 of the children had SCT, and 10 of the children were normal healthy children. Participants with SCD and SCT were recruited from the Paediatric Unit at Sur Regional Hospital (SRH). Normal healthy children without SCD were recruited from local schools in the city of Sur. The participants were matched in age and gender with the SCD and SCT participants. Participants were admitted to the Paediatric unit at SRH for testing. During their period of admission, body composition and anthropometric measurements were taken. Electrocardiograph (ECG) and resting blood samples were measured, and the exercise test was conducted at the cardiology unit. Exclusion criteria included any participants with a history of blood transfusion, stroke, serious cardiac arrhythmias, vaso-occlusion, and hypertension. The procedures and
protocol of this study were reviewed and approved by the SRH Ethics Committee. Each participant and their parents signed a written, informed consent form after reading a detailed explanation and receiving a verbal description of the procedures and protocols of the study.

Design

Body Composition

Height was measured using a fixed digital stadiometer to the nearest 0.01 cm. Body mass was measured using a digital electronic clinical scale to the nearest 0.05 kg with subjects wearing light clothing and no shoes. Body Mass Index (kg/m$^2$) was calculated. Body fat percentage was calculated using the Slaughter equation for children (Slaughter et al., 1988) (see Chapter 2.3).

Treadmill exercise test

A modified Bruce protocol was used as a sub-maximal exercise stress test. This protocol has been widely used with high-risk and elderly individuals (Heyward, 2002). Time was recorded from the first minute of the test until the subject completed all stages of the test or was unable to continue. The test was terminated if a subject exhibited symptoms of an inability to continue, such as ECG abnormalities, heart rate above 175 bpm or signs of exhaustion. Heart rate and blood pressure were measured throughout the exercise. A CardioSys-series 2000 treadmill with Cardamax-fx 2111-ECG machine was used for this test.
Multi-stage fitness test (MFT)

The 20 m MFT was administered in a sports hall using the original protocol (Leger & Lambert, 1982) and as described in the general methods. The test score achieved is the number of 20 m laps completed before the subject either withdraws voluntarily from the test or fails to be within 2 m of the end lines on two consecutive laps.

Haematological indices

Following an overnight fast, a resting venous blood sample was taken following rest in a supine position for 15 minutes. Further blood samples were collected immediately post-exercise and 10-minutes after the treadmill test. For each sample, the skin was cleaned with isopropyl alcohol or iodine, and the staff nurse from the paediatric unit drew venous blood by venipuncture from an anterior cubital vein. The blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes and stored at 4°C until undergoing analysis at SRH laboratory. Complete blood count (CBC) was conducted using Automatic Haematology Analyser (SF 3000, Sysmex, UK): the Hb, Hct and reticulocyte count were determined.

Maturation and physical activity assessment

Maturation in children with SCD, SCT and normal healthy children was assessed with a non-invasive method using parents’ heights (Khamis & Roche, 1994) (see Chapter 2.5). The Leisure-Time Exercise Questionnaire (LTEQ: Godin & Shephard, 1985) was completed by participants to assess physical activity levels (see Chapter 2.4).
**Statistical analysis**

Data were analysed using SPSS version 17 (SPSS Inc., Chicago, USA). Mixed between-within subjects ANOVA was used to detect the main effects and interactions among groups for heart rate, blood pressure, Hb, RBCs, WBCs, Hct, and reticulocytes. One-way ANOVA was used to compare means among groups for all of the anthropometrics measures and the multistage fitness test. Tukey’s *post hoc* testing was used to locate any significant differences among groups. Physical activity data was analysed using a non-parametric Kruskal–Wallis test. All data are presented as mean (SD). Significance was accepted at $P < 0.05$.

**4.3. Results**

**Anthropometric measurements, body composition and maturation**

Table 4.1 indicates that there were no significant differences among normal healthy children and children with SCD and SCT in age and body fat percentage ($P > 0.05$). ANOVA showed significant differences in body mass and body height among the groups ($P < 0.05$). *Post hoc* tests indicated lower mean values ($P < 0.05$) in children with SCD, compared with SCT subjects for body mass. Mean height was greater in normal healthy and SCT children, compared with SCD children ($P < 0.05$). Body mass index was lower in children with SCD, compared with SCT ($P < 0.05$). Maturation assessment indicated that five of the children with SCD were maturing on time, and three were late maturers. Five of the children with SCT were advanced maturers, and four were maturing
on time. In normal healthy children, five were advanced maturers, while the other five were maturing on time.

Table 4.1: Subject characteristics. Values are mean (SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control group (n=10)</th>
<th>Sickle cell trait (n=9)</th>
<th>Sickle cell disease (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>14.3 (1.0)</td>
<td>13.7 (0.9)</td>
<td>13.2(1.2)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>38.6 (6.7)</td>
<td>48.6 (12.1)</td>
<td>31.7 (9.1) #</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>149.6 (9.8)</td>
<td>154.6 (6.0)</td>
<td>136.7 (13.8)*</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>17.2(2.0)</td>
<td>20.2 (4.2)</td>
<td>16.6 (1.4) #</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>30.5 (10.9)</td>
<td>24.4 (11.2)</td>
<td>23.9 (6.7)</td>
</tr>
</tbody>
</table>

*Significantly lower than normal healthy children and SCT children ($P < 0.05$).

# Significantly lower than SCT children ($P < 0.05$).

**Physical activity questionnaire**

Using a Kruskal–Wallis test, physical activity level was significantly different across the study populations ($H (2) = 16.606, P < 0.05$). Overall inspection of the mean ranks for the groups suggested that normal healthy children had the highest mean ranks for physical activity levels (21.0), with SCT children reporting intermediate mean ranks (17.3), while SCD children reported the lowest (5.6). One-way between-groups ANOVA with *post hoc* analysis was applied to locate the differences among groups. The results indicated that the normal healthy children had a significantly higher physical activity
level than the SCT and SCD children ($P < 0.05$). Furthermore, the SCT children had a significantly higher physical activity level than the SCD children ($P < 0.05$).

Multi-stage fitness test

Figure 4.1 indicates that one-way ANOVA with a post hoc comparisons test indicated significant differences in multi-stage fitness test performance among the three groups ($P < 0.05$). Normal healthy children had a higher estimated VO$_2$max than SCT and SCD children ($P < 0.05$), while SCT children had higher values than SCD ($P < 0.05$). The effect size calculated using eta squared indicated a large effect size (0.758).

Figure 4.1: Mean (SD) multistage Fitness Test for normal healthy, SCT and SCD children. * Significantly different MFT performance, compared with normal ($P < 0.05$).

# Significantly different MFT performance, compared with SCT ($P < 0.05$).
Heart rate response during treadmill exercise

There were no significant differences among the three groups in resting heart rate ($P > 0.05$). All 27 subjects did not complete the seven stages of treadmill test. Figure 4.2 indicates heart rate over time; heart rate increased steadily throughout the test, and there was significant effect for time ($P < 0.05$) with a large effect size (eta squared = 0.983). The interaction effect was not significant ($P > 0.05$). There was a significant main effect for group ($P < 0.05$) with a medium effect size (eta squared = 0.459). ANOVA with post hoc test indicates that normal healthy children had lower mean heart rates than both SCD and SCT children.

Figure 4.2: Mean (SD) heart rate across time in normal healthy, SCT and SCD children.

*Main effect for group, significantly higher mean heart rate in SCD and SCT than normal healthy children, ($P < 0.05$).
Blood pressure responses

**Systolic blood pressure**

Systolic blood pressure increased from pre- to immediately post-exercise and then decreased again during recovery (time effect, \( P < 0.05 \)) with a large effect size (eta squared= 0.802) as well as a significant main effect for group (\( P < 0.05 \)) with a large effect size (eta squared= 0.929). There was also a significant interaction among the groups over time with a small effect size (eta squared= 0.182). *Post hoc* tests showed that both SCD and SCT subjects had higher systolic blood pressure than normal healthy subjects (\( P < 0.05 \)).

![Graph showing systolic blood pressure](image)

Figure 4.3: Mean (SD) systolic blood pressure before, immediately after and 10 min after treadmill exercise in normal healthy, SCT and SCD children. * Main effect for group, significantly higher in SCD and SCT subjects during exercise than in normal healthy children, (\( P < 0.05 \)).
**Diastolic blood pressure**

Diastolic blood pressure also increased from pre- to immediately post-exercise and then decreased again during recovery (time effect, $P < 0.05$) with a medium effect size ($\eta^2 = 0.596$). There was no significant interaction among groups ($P > 0.05$) and no significant difference in diastolic blood pressure among the three groups ($P > 0.05$).

![Figure 4.4](image-url)  
**Figure 4.4:** Mean (SD) diastolic blood pressure before, immediately after and 10 minutes after treadmill exercise in normal healthy, SCT and SCD children
Haematological measurements

Haemoglobin

ANOVA showed that there was a significant main effect for time in Hb concentration (see Figure 4.5) ($P < 0.05$) with a small effect size ($\text{eta squared} = 0.333$). There was also a significant effect for group in Hb levels ($P < 0.05$) with a medium effect size ($\text{eta squared} = 0.600$). Post hoc calculation reported significantly lower Hb concentration in SCD subjects ($P < 0.05$). The interaction among the groups over time was not significant ($P > 0.05$).

![Figure 4.5: Mean (SD) Hb before, immediately after and 10 minutes after treadmill exercise in normal healthy, SCT and SCD children. * Main group effect, significantly lower in SCD than SCT and normal healthy children, ($P < 0.05$).](image)

Normal
SCT
SCD

Figure 4.5: Mean (SD) Hb before, immediately after and 10 minutes after treadmill exercise in normal healthy, SCT and SCD children. * Main group effect, significantly lower in SCD than SCT and normal healthy children, ($P < 0.05$).
**Haematocrit**

In Hct (see Figure 4.6), there was an effect for time \((P < 0.05)\) with a small effect size \((\text{eta squared}= 0.285)\). ANOVA also indicated effect for group \((P < 0.05)\) with a medium effect size \((\text{eta squared}= 0.676)\), where *post hoc* testing showed that the SCD group had lower Hct than both SCT and normal healthy children \((P < 0.05)\). There was no interaction effect in groups with time \((P > 0.05)\).

**Figure 4.6**: Mean (SD) Hct before, immediately after and 10 minutes after treadmill exercise in normal healthy, SCT and SCD children. Main effect for group, SCD significantly lower than SCT and normal healthy children, \((P < 0.05)\).
**Red blood cells**

Red blood cells did not change over time in all three groups but was different among groups (main effect for group, $P < 0.05$) with a medium effect size (eta squared= 0.461). Applying *post hoc* testing, SCD had significantly lower RBC than other two groups ($P < 0.05$). ANOVA did not report any significant effect for time in RBC and no significant interaction effect among groups over the time ($P > 0.05$).

![Red blood cells graph](image)

**Figure 4.7:** Mean (SD) RBC before, immediately after and 10 minutes after treadmill exercise in normal healthy, SCT and SCD children. *Main group effect, significantly lower in SCD than SCT and normal healthy children, ($P < 0.05$).*
**Reticulocytes (percentage of total RBCs in blood)**

ANOVA revealed a main effect for time for reticulocyte percentage ($P < 0.05$) with a medium effect size (eta squared= 0.674). ANOVA also indicated that there was a main effect for group ($P < 0.05$) with a large effect size (eta squared= 0.936). There was also an interaction effect for reticulocytes among the groups over time ($P < 0.05$) with a medium effect size (eta squared= 0.493). *Post hoc* tests showed that SCD children showed a higher proportion of RBCs as reticulocytes at all time points and had a different reticulocyte response to exercise than SCT children and normal healthy children ($P < 0.05$).

![Graph](image)

Figure 4.8: Mean (SD) reticulocyte percentage before, immediately after and 10 minutes after treadmill exercise in normal healthy, SCT and SCD children. *Main group effect, significantly higher in SCD than SCT and normal healthy children ($P < 0.05$).
**White blood cells**

A main effect for time was found in WBC \((P < 0.05)\) with a large effect size (eta squared= 0.753). There was a main effect for group in WBC \((P < 0.05)\) with a small effect size (eta squared= 0.316). *Post hoc* testing revealed a higher WBC concentration in SCD, compared with SCT and normal healthy children \((P < 0.05)\). ANOVA showed there was no interaction effect among groups over time \((P > 0.05)\).

![Figure 4.9: Mean (SD) WBC before, immediately after and 10 minutes after treadmill exercise in normal healthy, SCT and SCD children. *Main group effect, significantly higher in SCD than SCT and normal healthy children \((P < 0.05)\).](image-url)
4.4. Discussion

The present study was designed to evaluate the cardiopulmonary fitness of children with SCD and SCT in comparison with normal healthy children. The results of this study confirmed that normal healthy children had a significantly higher estimated VO$_2$max than SCT and SCD children ($P < 0.05$), with SCD children achieving the lowest VO$_2$ max. The mean heart rate in SCD and SCT was significantly higher during sub-maximal exercise than normal healthy children ($P < 0.05$). Sickle cell trait and SCD children had a significantly higher blood pressure in response to exercise than normal healthy children ($P < 0.05$).

In this study, children with SCD had significantly lower aerobic fitness levels, compared with the other two groups, as they reached exhaustion earlier. The results of the multi-stage fitness test showed that normal healthy children had a higher estimated VO$_2$max than SCT and SCD children ($P < 0.05$), while SCT children had higher values than SCD ($P < 0.05$). Other studies in children reported similar results, indicating that children with SCD have lower VO$_2$ max than SCT and normal healthy children (Braden et al., 1996; Pianosi et al., 1991), although the mean VO$_2$ max values in all groups were higher than those in the present study, probably due to differences in lifestyle and physical activity habits as well as differences in the methods used in measuring the VO$_2$ max, as both previous studies used direct measures. In the present study, children with SCD had lower physical activity scores than normal healthy and SCT children. Physical activity level has been proved to enhance physical fitness and to improve quality of life in
the general population (Balducci et al., 2009; Colberg & Grieco, 2009). It is likely that
the lower physical activity of SCD children in the present study contributed to their lower
fitness presented as lower VO₂ max. Mota et al. (2002) studied 254 male and 240 female
subjects aged between 8 and 18 years using the multistage shuttle run test, with estimated
VO₂ max for normal male children of 46.9 ± 4.7 ml/kg/min, which is higher than the
values of mean VO₂ max (32.9 ± 4.1 ml/kg/min) for normal healthy children in this
study. One possible explanation for these results could be the differences of the group
samples, as the sample of the present study included children aged 12 to 15, and it has
been suggested that VO₂ max peaks around the age of 18 in healthy boys (Armstrong &
Welsman, 2000).

The lower VO₂ max in sickle cell children may be explained in term of blood
components that contribute as a key determinant of blood oxygen-carrying capacity. As
Hb is the main vehicle for carrying oxygen to the body tissues, it is essential to assess Hb
concentration as a major determinant of VO₂ max. Bassett and Howley (2000) stated that
increasing a subject’s volume of total RBCs increases their oxygen-carrying capacity. In
the present study, Hb concentration, RBC and Hct values were lower in SCD subjects.
The lower [Hb], RBC count and Hct mean that there is a reduced ability of blood to carry
oxygen to tissues in the SCD and SCT groups, thus reducing VO₂ max, assuming other
factors such as increasing cardiac output remain insufficient to compensate for the lower
oxygen-carrying capacity of blood. Srinivasan et al. (1999) documented that during
exercise testing, the exercise performance of SCD patients who exhibit mild or less
severe complications was limited by cardiovascular mechanisms. Haywood (2009)
suggested that although individuals with low Hb levels exhibit a response indicative of increased cardiac output during exercise, they still display ECG changes indicating ischaemia. The findings of the present study suggest that SCD children had greater overall cardiac output, higher heart rate and higher blood pressure, in an attempt to compensate for the lower oxygen in blood. As physical exertion increases the body’s oxygen demand, and given that there is a profound defect in oxygen supply to tissue in SCD, exhaustion and even hypoxia of tissue may result earlier than normal, contributing to the lower fitness in this population. At rest, the reticulocyte (immature RBCs) percentage of total RBCs in children with SCD was higher than in the other two groups, which can be explained by an above-normal demand for RBC production. During post-exercise and recovery in SCD children, the reticulocytes percentage was higher than the other two groups. The higher reticulocytes in SCD could be explained by the constant haemolysis of RBC leading to increased production of reticulocytes from the bone marrow.

During exercise, SCD children’s cardiac output and muscle blood flow increase and remain high relative to the work intensity, and consequently exhaustion occurs early (Covitz et al., 1983). Srinivasan et al. (1999) also documented that SCD patients with frequent crises or history of ACS were limited by pulmonary mechanisms, which Haywood (2009) explained by pulmonary insufficiency in terms of restrictive lung disease and pulmonary acidosis. Since in this study we had a relatively healthy subset of SCD patients with no significant pulmonary abnormalities, it is likely that the cardiovascular limitations explain the lower exercise capacity.
The lower VO$_2$\text{max} in SCD children in this study could also be explained in part by the smaller body size and the earlier stage of skeletal maturation in this group. Five of the eight SCD children in this study were found to be maturing on time, while three of them were late maturers. In contrast, all of the SCT and normal healthy children were maturing on time or were early maturers. A combination of the slightly younger chronological age (although this was not significant), smaller body size and slightly later skeletal maturation of some of the SCD children might explain some of the differences in performance. Studies in SCD demonstrated a relationship between delays in sexual and skeletal maturation in children and physical performance (Singhal et al., 1994). It has been reported that factors such as motor skills (Jones et al., 2000) or the economy and efficiency of running (Ebbeling et al., 1992) increase with growth and maturation. Wethers (1989) reported delayed growth in SCD children at the ages of 3 to 14 years old, with weight being more affected than height and with an associated delay in bone age by early adolescence. Wethers (1989) suggested that endocrine function and nutritional factors may play a prime role in growth delay for SCD children. The energy demands of the bone marrow for RBC production compete with the demands of a growing body (Silva & Viana, 2002). Therefore, variation in skeletal maturation observed in the present study may play a role in explaining the variation in the 20 m shuttle run test. Differences in the rate of growth are mainly responsible for individuals’ differences in physical performance in paediatric age groups (Malina et al., 2004). The lower level of skeletal maturity and lower body size may contribute to the poorer performance in children with
SCD in the present study. Unfortunately, no supporting information relating to nutritional habits and endocrine function was collected.

The BMI of all participants in the present study was low to normal, compared with average healthy boys’ data from WHO (de Onis et al., 2007). The present study findings revealed that SCD children had lower body mass and BMI, compared to SCT subjects. However, SCD and SCT children had similar body fat percentages, which were both lower than the body fat percentages of healthy subjects. These findings disagree with those reported by Moheeb et al. (2007) in children with SCD from Oman. Moheeb et al. (2007) documented that children with SCD had a higher body fat percentage than normal healthy children, due to the lack of physical activity. Comparison of the two studies’ results may be affected by differences in sample age, as the children in the present study were older. Also, comparison may be affected by the socioeconomic status and lifestyle of the children, as Moheeb et al. (2007) recruited participants from the capital city area, while in this study the subjects were recruited from a town with lower socioeconomic status. In the previous chapter (Chapter 3), anthropometric data collected for children recruited from the capital city showed similar results to those reported by Moheeb et al. (2007), possibly indicating the effect of socioeconomic status. However, most studies report a lower body fat percentage and BMI in children with SCD (Barden et al., 2002; Mukherjee & Gangakhedkar, 2004; Silva & Viana, 2002). In contrast, some studies in adult subjects with SCD indicated a relatively high prevalence of obesity and a risk of developing chronic heart disease (Pells et al., 2005; Woods et al., 2001). Research in SCD children has reported a lower weight-to-height ratio in children with SCD,
suggesting that they are in a hypermetabolic state (Fung et al., 2001; Hibbert et al., 2005; Singhal et al., 2002). The high metabolic rate in SCD patients is associated with increased erythrocyte production, cardiac work rates and protein metabolism (Salman et al., 1996). Moreover, patients with SCD may have reduced appetite associated with regular pain crises, leading to low nutritional intake (Malinauskas et al., 2000). In SCD, the reduction in physical activity level may be the result of a combination of these factors, given that SCD children recorded lower physical activity levels in the present study, compared with other groups.

As expected, WBC levels were different among the normal healthy subjects, SCT children and SCD children, with higher WBC ($P < 0.05$) in the SCD group than in the other two groups. It is well documented that patients with SCD have chronic inflammation, elevating their WBC (Akohoue et al., 2007). The sickle red cell is an irritant that provokes an inflammatory response, as it obstructs blood flow. Tissues suffer hypo-perfusion and are exposed to inflammatory cytokines, growth factors and other actions of the activated inflammatory cells (Platt 2000). Darbari et al. (2006) stated that infections accounted for about 18% of SCD deaths. For all of the reasons above, the role of inflammation and inflammatory markers in the pathophysiology of SCD would be interesting to study further.

A limitation of this study might lie with the non-invasive measure for maturity. This is the first study to employ this measure in children in Oman, which may affect the validity of comparison with children in the original assessment in the absence of
population-specific reference curves for comparison (Khamis & Roche, 1994). However, delayed maturation is an effect of SCD, and therefore the finding that children with SCD have different exercise capacity, compared with normal healthy children of the same chronological age suggests that this method is sensitive to differences in maturation in this population.

Although there are no significant age differences among the groups, SCD children were different in their anthropometric and haematological measures than SCT and normal healthy children. SCD children had significantly lower height compared to normal healthy and SCT children. They also displayed lower Hct, Hb, and RBC count than the SCT and normal healthy group. The WBC count was significantly higher in the SCD group, compared to the normal healthy and SCT groups. These results could be implicated as a potential explanation for these individuals’ lower exercise capacity and provide a justification for them to engage in moderate-intensity exercise. The lower Hb concentration and lower RBCs reduce the oxygen-carrying capacity to tissue, which will lead to early exhaustion. Other suggested causes are late maturation and lack of regular exercise. The SCD children were found to be less active than normal healthy children, which might be due to complications associated with the condition, delay in growth patterns, insufficient energy intake and other socioeconomic and environmental factors, such as hot weather, that might cause dehydration, which increases the rate of sickling of RBCs (Bergeron et al., 2004).
In conclusion, the results of the present study suggest that children with SCD have limited cardiopulmonary fitness in comparison with normal healthy children, as these children had a lower estimated VO$_2$max and higher mean heart rate and blood pressure during sub-maximal exercise. In addition, the results indicated that WBC count was higher at all time points in SCD children compared to other groups indicative of a chronic inflammatory state. Given that these children might have a chronic inflammatory state, an important key to develop a better understanding of the relationship between SCD and exercise is investigating the effect of exercise in inflammatory markers in this population.
5. EFFECT OF AN ACUTE BOUT OF EXERCISE IN IL-6 CONCENTRATIONS IN OMANI CHILDREN WITH SICKLE CELL DISEASE

5.1. Introduction

Patients with SCD are hypermetabolic and show several abnormalities of the immune system such as high WBC counts and elevated serum levels of acute phase proteins such as CRP and pro-inflammatory cytokines such as IL-6 (Hibbert et al., 2005). Indeed, in the study described in Chapter 4, children with SCD had significantly greater WBC counts than SCT and normal healthy children. Hypermetabolism, an abnormal increase in the metabolic rate, is generally associated with cytokine driven inflammation in cases of injury or infection. In children with SCD, hypermetabolism was strongly related to chronic inflammation demonstrated by elevated levels of CRP and WBC count (Hibbert et al., 2005).

Deformed RBCs and their abnormal contents act as irritants to the vascular endothelium and surrounding tissue. The tissue becomes under-perfused and exposed to inflammatory cytokines, GFs and the actions of the activated inflammatory cells (Platt, 2000). Platt (2000) indicated that although all individuals with SCD have the same mutant globin gene, there is a wide range of clinical severity, as the phenotype could be affected by several other factors, such as the environment and the patient’s immune and nutritional status. In general, patients with SCD show elevated baseline leukocytes,
although this elevation varies among individuals. The variation in baseline leukocyte count among SCD is predictive of the severity of the disease (Platt, 2000).

Inflammation plays a significant role in the clinical manifestations of SCD. It is therefore important to clarify the response of inflammatory factors to exercise, especially given that changes in some inflammatory mediators, such as IL-6 and CRP, may influence the occurrence of vasco-occlusive episodes by increasing adhesion of erythrocytes to the endothelium (Pathare et al., 2003). Pathare et al. (2003) suggested two mechanisms by which cytokines, including IL-6, affect microvascular occlusion in SCD. The first mechanism is by activating the endothelial cells’ expression of adhesion molecules and facilitating adhesion receptor interaction with the corresponding ligand on the surface of the blood cell. The second mechanism is by increasing the coagulation mechanism by decreasing anticoagulant molecules. However, to date, no studies have been published that examine the inflammatory response to exercise in children with SCD. As IL-6 is an important inflammatory cytokine, playing a major role in the inflammatory response, and as it is the first cytokine released into the circulation in response to exercise (Pedersen, 2006), measuring the influence of exercise on IL-6 in children with SCD might aid the assessment of the role of exercise in this population.

The aim of this study was to determine, for the first time in children, whether a single bout of exercise elicits changes in IL-6 concentrations in SCD patients and how this compares with a normal healthy group.
Hypothesis

Given the effect of sickle RBCs on the vascular endothelium, it is expected that at rest the concentration of the inflammatory cytokine IL-6 will be higher in SCD children than normal healthy children. Considering the fact that exercise increases release of IL-6, it is expected that both the SCD and normal healthy groups will have higher IL-6 concentrations following a single bout exercise and that there will be a difference in the IL-6 response between the two groups.

5.2. Methods

Subjects

The participants were Omani male children aged 14 to 15 years. One group were normal healthy children with normal Hb (HbAA, n=7). The other group of children had SCD (HbSS, n=7). Participants with SCD were recruited from the Paediatric Unit and Genetic Disease Unit at SRH. Normal healthy subjects were recruited from local schools in the city of Sur. Normal healthy participants were matched in age to the SCD children. Participants were admitted to the Cardiology Unit at SRH for testing. A detailed written informed consent was obtained from each participant prior to the procedure. During their period of admission, body composition and anthropometric measurements were conducted, a physical activity questionnaire was completed and parents’ height and body mass were recorded, either while they were in the hospital or by visiting their home. The
parents’ data were obtained in order to evaluate the predicted maturity of the subjects. An exercise test was conducted with blood samples taken immediately pre-, immediately post- and 24 hours post-exercise. Electrocardiograph was performed during exercise for the safety of the participants. Any patient with a history of blood transfusion, stroke, hypertension or serious cardiac arrhythmia was excluded from the study. A physician and staff nurse were present during all testing. The study protocol was approved by the Medical and Ethical Research Committee at SRH.

Design

Anthropometric measures

Subject height and body mass was measured at the beginning of each experimental day. Skinfold measurements were taken at two sites (Subscapular + Triceps) using Harpenden Skinfold calipers (John Bull, England). Body fat percentage was then calculated using the Slaughter equation for male children (Slaughter et al., 1988).

Physical activity assessment

The LTEQ (Goding & Shephard, 1985) was completed by participants to assess physical activity levels (See chapter 2.4).

Treadmill exercise test

Applying the modified Bruce protocol, subjects were required to walk on the treadmill until exhaustion or until reaching a point at which they could not continue
walking or until their heart rate exceeded 175 bpm. Heart rate was measured every three minutes during the exercise test. Subjects walked with the ECG attached to identify any cardiac abnormalities during the test.

**Haematological indices**

Following an overnight fast, a resting venous blood sample was taken following rest in a supine position for 15 minutes. The skin was cleaned with isopropyl alcohol or iodine, and staff nurse drew venous blood by venipuncture from the anterior cubital vein. The blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes.

Pre- and post-exercise blood samples were collected for the measurement of a CBC, Hb and Hct. These samples, as well as those taken at 24 hours, were taken to provide information on the degree to which the exercise produced an inflammatory response, as measured by circulating IL-6 concentrations. After immediate analysis of CBC in the laboratory (SF 3000, Sysmex, UK), plasma was separated and stored at –40º C until it was transported to Bath for the analysis of IL-6. IL-6 was analysed using ELISA kit (Quantikine, Abingdon, UK).

**Statistical analysis**

Independent Sample t-tests were applied to detect differences in mean values between the two groups for the baseline measures such as body mass, height, BMI, RBC, WBC, Hb and Hct. The results of the t-test were accepted to be significant at $P < 0.05$. Two-way repeated measures analysis of variance (ANOVA) was applied to determine
differences between the normal healthy children and SCD children for IL-6 and heart rate. Data are presented as means (SD). A Mann–Whitney U test was used to detect differences in the levels of physical activity between the SCD children and the normal healthy children.

### 5.3. Results

**Anthropometric and body composition measurements**

There were no significant differences between normal healthy children and children with SCD in age and body mass ($P > 0.05$). Likewise, the data showed no significant differences in body fat and BMI between the two groups ($P > 0.05$). As Table 5.1 shows, the mean values for height were significantly greater in the normal healthy children, compared with SCD children ($P < 0.05$).

**Table 5.1**: Mean (SD) of age and anthropometric measures

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal group (n=7)</th>
<th>Sickle cell disease (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>14.9(0.15)</td>
<td>14.6(0.45)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>46.2(13.0)</td>
<td>40.0(7.8)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>158.0(9.5)</td>
<td>146.1(9.4)*</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>18.3(4.5)</td>
<td>18.5(2.3)</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>26.7(6.7)</td>
<td>22.3(3.0)</td>
</tr>
</tbody>
</table>

*Significant differences at ($P < 0.05$).
Haematological measures

Table 5.2 shows significant differences between the two groups in resting RBC, WBC, Hb levels and Hct. Children with SCD have significantly lower RBC count, Hb and Hct, compared with the normal healthy children ($P < 0.05$). Resting WBC was higher in the SCD children than in the normal healthy children ($P < 0.05$).

Table 5.2: Mean (SD) of RBC, WBC, Hb and Hct

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal group (n=7)</th>
<th>Sickle cell disease (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC ($\times 10^{12}$ cell/L)</td>
<td>4.9(0.36)</td>
<td>4.0(0.54) *</td>
</tr>
<tr>
<td>WBC ($\times 10^{9}$ cell/L)</td>
<td>4.4(0.84)</td>
<td>8.9(2.4) *</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>12.5(0.63)</td>
<td>9.6(1.0) *</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>37.5(1.4)</td>
<td>28.0(2.5) *</td>
</tr>
</tbody>
</table>

*Significant differences at ($P < 0.05$).

Physical activity and maturation

Physical activity level was significantly different across the study populations ($U = 0.00$, $P < 0.05$). Overall inspection of the mean ranks for the groups suggested that normal healthy children had the highest mean ranks for physical activity levels (11.00), while SCD children reported the lowest (4.00). The results from the maturation assessment indicate that four of the normal healthy children were maturing on time, while two were advanced, and one child was a late maturer. On the other hand, five of the children with SCD were maturing on time, and two were late maturers.
Figure 5.1: Mean (SD) heart rate in SCD children and normal healthy children during treadmill exercise test. * Main effect for group, significantly higher in SCD ($P < 0.05$).

All of the normal healthy children ($n = 7$) completed 15-minute of the exercise test, while all SCD children ($n = 7$) stopped at minute 12; five children reported an inability to complete the test due to exhaustion and two were stopped by the physician due to reaching the maximum recommended heart rate of 175 bpm (Mani et al., 2005). ANOVA revealed a main effect for time ($P < 0.05$) with a very large effect size ($\text{eta}^2 = 0.997$). The interaction effect between groups was significant ($P < 0.05$). The group effect was also significant ($P < 0.05$) with a medium effect size ($\text{eta}^2 = 0.663$). The t-test revealed higher heart rates for the SCD children, compared to normal healthy children ($P < 0.05$) through all time points except for Minute 9.
Interleukin-6

Figure 5.2: Mean (SD) plasma IL-6 for SCD children and normal healthy children at rest, post exercise and 24 hours after exercise. * Main effect for group, significantly higher in SCD ($P < 0.05$).

There was no significant main effect for time ($P > 0.05$). The results indicated no interaction effect in groups with time ($P > 0.05$). Sickle cell disease children showed higher mean IL-6 concentrations than normal healthy children (main effect for group; $P < 0.05$) with a medium effect size (eta squared = 0.559).
5.4. Discussion

The present study was designed to determine the effect of one bout of exercise on IL-6 concentrations in SCD subjects, compared with normal healthy subjects. Several studies in adults indicate that acute exercise induces an inflammatory response similar to that associated with infections or mild trauma (Fernandes et al., 2011; Tokgozoglu, 2009). However, to date, no studies have been published investigating the response of IL-6 to exercise in children with SCD. The main finding of this study showed that SCD children had higher mean IL-6 than normal healthy children, while there was no difference in IL-6 concentration in response to exercise in both groups.

Plasma IL-6 concentration was measured for both normal healthy and SCD children at three time points; at rest, post-exercise and 24 hours after exercise. The results showed that children with SCD had higher mean IL-6 concentrations at all three time points. Several studies have shown that, at rest, IL-6 levels are elevated in patients with SCD (Greenough, 2004; Makis et al., 2000; Pathare et al., 2004). The baseline IL-6 results of the present study contradict those found by Barbeau et al. (2001), who indicated that the baseline IL-6 in SCD adult subjects was similar to those of normal healthy adults. According to Barbeau et al. (2001), only one other study in the literature had similar IL-6 baseline results (Gradio-Gonzalez et al., 1998). The higher baseline IL-6 concentration can be explained by the fact that SCD patients are in a continuous inflammatory state owing to persistent damage to the tissues caused by the sickled RBCs (Platt, 2000). Aslan & Freeman (2007) stated that tissue ischemia and vaso-occlusive
crisis may induce a continuous inflammatory response in SCD. One of the important features of inflammation is the migration of leukocytes from the circulation to the vascular endothelium. This process is induced by pro-inflammatory cytokines such as IL-6, which is secreted by tissue macrophages and is elevated in plasma of SCD patients (Aslan & Freeman, 2007). This process may explain the high mean baseline IL-6 concentrations in SCD subjects indicated in the present study.

The results of the present study indicated that both SCD and normal healthy children had no significant change in IL-6 concentrations in response to exercise. Pedersen (2006) has reported in a study in healthy subjects that IL-6 is the first cytokine to be released into the circulation in response to exercise and that its concentration increases during exercise. Interleukin-6 has also been reported to remain elevated 24 hours after exercise after maximal and sub-maximal work in healthy subjects (Edwards et al., 2006; Sprenger et al., 1992). These findings are in disagreement with the findings reported in the present study, as normal healthy children showed no increase in IL-6 concentration post-exercise and 24 hours after exercise (see Figure 5.2). The magnitude of the IL-6 response to exercise depends on the exercise intensity, duration, mass of functioning muscle and endurance capacity of the subject (Pedersen, 2006). The absence of an exercise response in both SCD and normal healthy children presented in this study suggests that the exercise duration and intensity may not have been sufficient to elicit a significant IL-6 response. To our knowledge, only one other study has reported the effect of exercise on IL-6 concentrations in subjects with SCD. Barbeau et al. (2001) investigated adult subjects, while the present study investigated children. Barbeau et al.
(2001) reported no significant change in IL-6 concentration in SCD subjects after a 30-minute treadmill walking exercise, which is in agreement with the findings of the results for SCD subjects in the present study. Barbeau et al. (2001) measured blood IL-6 concentrations for three constitutive days after the test and noticed no change in IL-6 concentrations over the time. The results of the present study agree with those reported, as SCD subjects showed no significant change in IL-6 concentrations post-exercise and 24 hours after exercise.

Anthropometric, body composition and haematological measures were determined in order to evaluate variations between the groups, and the heart rate was used by the physician to determine whether it was necessary to terminate the exercise for the individual subject. Earlier studies conducted to compare physical fitness and cardiovascular responses to exercise between SCD children and normal healthy children (Chapters 3 and 4, respectively) indicated similar anthropometric and haematological results to those reported by the present study. The results of the present study also indicated that SCD children reported a higher heart rate response to exercise than normal healthy children. The higher heart rate might be related to the anaemic state of this population (lower RBC and Hb as reported in Table 5.2). Increasing the heart rate might suggest a compensatory mechanism for the lower oxygen-carrying capacity of blood. These results are in agreement with those reported by Moheeb et al. (2007) and Balayssac-Syransy et al. (2011), who reported that SCD subjects exhibited higher heart rate responses to sub-maximal exercise than normal control subjects. Alternatively, Adebayo et al. (2002) stated that in response to mild-exercise, adolescent SCD patients
had a similar heart rate response to the controls, and suggested that SCD patients compensate for the high oxygen demands related to exercise by increasing their cardiac output via increasing the stroke volume and the cardiac index, while the heart rate response was similar to the normal controls. In the present study, none of the SCD children completed the exercise test, due to exhaustion or exceeding the highest recommended heart rate. Balayssac-Syransy et al. (2011) performed a study in adult males, which reported that subjects with SCD exhibited a greater cardiopulmonary stress (marked by increased ventilation and heart rate), compared to normal subjects in response to a 20-minute cycling exercise test. The findings of Balayssac-Syransy et al. (2011) might explain the early exhaustion reported by SCD subjects in the present study.

In conclusion, children with SCD exhibited higher baseline IL-6 concentrations than the normal healthy children, suggesting a persistent inflammatory state. The study also indicated no change in IL-6 concentrations in response to exercise in both SCD and normal healthy children. The effect of one bout of exercise on IL-6 responses in subjects with SCD still needs further assessment, since the exercise used in the present study does not appear to have been sufficiently demanding.
6. EFFECT OF EXERCISE ON POSTPRANDIAL LIPAEMIA IN CHILDREN WITH SICKLE CELL DISEASE

6.1. Introduction

High triacylglycerol (TAG) plasma levels have been reported to result in endothelial dysfunction in healthy subjects (Hyson et al., 2003; Jagla & Schrezenmeir, 2001; Vogel et al., 1997). Endothelial function is impaired in SCD patients (Blum et al., 2005; Ergul et al., 2004; Hammerman et al., 1997), and therefore a better understanding of TAG concentrations in this population is important. Adults with SCD exhibit higher TAG and lower cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) concentrations than healthy subjects (Zorca et al., 2010). Furthermore, elevated serum TAG levels in SCD patients are associated with elevated markers of haemolysis, inflammation and endothelial activation, indicating an association between TAG levels, vascular dysfunction and increased rate of haemolysis (Seixas et al., 2010; Zorca et al., 2010), as well as painful crisis (Buchowski et al., 2007; Erasmus et al., 1990). Belcher et al. (1999) indicated that despite lower serum cholesterol concentrations in SCD, LDL from patients with SCD is more prone to oxidation, resulting in the release of free radicals and oxidative stress that contributes to subsequent injury to the endothelium.

Postprandial lipaemia is the presence of high concentrations of TAG in the blood after a meal (Herd et al., 2001). Postprandial lipaemia, rather than measurement of fasting TAG concentrations, is more informative when trying to understand changes in plasma
TAG concentrations and is considered to be a more useful approach to studying lipid metabolism (Patsch et al., 1992). The Columbia University Biomarkers Study showed that the postprandial TAG response in adolescents is similar to that in adults (Couch et al., 2000). Moreno et al. (2001) reported a significant increase in serum TAG in both obese and non-obese adolescents at 2 hours and 4 hours after an oral fat tolerance test. Physical activity bouts performed immediately before intake of high-fat meals have been shown to lower postprandial TAG in non-endurance-trained people (Herd et al., 2001). Furthermore, several studies have examined the influence of accumulated physical activity one day before consuming a high-fat meal on postprandial TAG concentrations with consistent findings indicating that accumulated physical activity is effective in lowering postprandial TAG concentrations (Barrett et al., 2007; Gill et al., 1998; Tolfrey et al., 2008; Tolfrey et al., 2012). Tolfrey et al. (2012) studied the effect of 30 and 60 minutes of jogging on postprandial TAG in 13-year-old adolescent boys, while Barrett et al. (2007) investigated the effect of continuous-exercise and intermittent-games activity in 15-year-old boys. The results of both studies indicated that, regardless of the exercise period, postprandial TAG was significantly lowered by exercise. However, to date, there are no studies examining the effect of exercise on postprandial TAG in a population with SCD.

The lipid profile can be used as an indicator for measuring the risk of developing cardiovascular, inflammatory and vaso-occlusive crisis in SCD patients. Therefore, the aim of this study was to investigate the effect of high-fat mixed meal on postprandial
TAG in children with SCD and to examine the effect of a single exercise bout on postprandial TAG.

**Hypothesis:**

Given the effect of a high-fat meal on plasma lipids and the effect of exercise in postprandial TAG in non-SCD adolescents, we hypothesised that postprandial TAG will increase after a high-fat meal in children with SCD and that prior exercise will reduce the size of the postprandial TAG response.

**6.2. Methods**

**Subjects**

Twelve male children with SCD participated in this study. Participants were recruited from the Haematology Unit and Children’s Health Unit at SQUH. They were admitted to the Paediatric unit at SQUH for testing. During their period of admission, body composition and anthropometric measurements were conducted. Electrocardiograph was performed during exercise, for the safety of the participants. Physical examination and medical history were carried out in the hospital for each patient before engaging in the study. Individuals with history of stroke, serious cardiac arrhythmias, vaso-occlusion, hypertension, blood transfusion or under any medication were excluded from the study. A physician and staff nurse were present during all testing. The study protocol was approved by the Medical and Ethical Research Committee at SQUH. Prior to
participation, study procedures and its potential risks and benefits were fully explained to the subjects and their parents before providing a written informed consent for participation in this study.

Design

Research Design

Each subject completed two 2-day trials: a brisk walking trial, and a rest trial. Two-day trials were used because skeletal muscle LPL activity is thought to peak 8 hours after exercise (Seip et al., 1997), and this enzyme facilitates the removal of TAG from the blood (Seip & Semenkovich, 1998). Trials were carried out in a randomised counter-balanced order with a 7-day gap between trials.

Preliminary Test

Before the main trials, all subjects performed a sub-maximal incremental treadmill test. A modified Bruce protocol was used to determine the walking speed for each subject. Subjects were encouraged to give their best effort during the brisk walking on the treadmill. Heart rates and ECG were monitored continuously during the test to detect any abnormalities. The test was designed to elicit 75% of the maximum recommended heart rate for this population.
**Main trials**

**First Day**

On the first day of each trial, the subjects reported to the laboratory at 0900. On arrival in the laboratory the subjects were asked to sit quietly for 10 minutes, and then a baseline resting blood sample was obtained. For the walking trial, the subjects performed three 10-minute bouts of treadmill brisk walking. A 10-minute rest interval followed each bout such that the total exercise bout lasted 50 minutes. Walking speed was determined by the cardiologist using the ECG as the point where the subject reached 75% of maximum recommended heart rate of 175 bpm for SCD population (Mani et al., 2005). For the rest trial, the subjects were asked to sit quietly and watch TV at the children’s hall of the hospital. On leaving the laboratory, the subjects were instructed to consume an early evening meal and to rest for the remainder of the evening. Children were asked to report their diet on the evening of day one of the first trial and to repeat the same diet on the evening of day one of the second trial.

**Second Day**

On the second day of each trial, the subjects reported to the laboratory at 0900 after a 10-hour overnight fast (no food or drink except water). The subjects rested for 10 minutes after their arrival at the laboratory. A cannula was then inserted into an antecubital vein, and a baseline blood sample was collected. The subjects then consumed (under supervision) a mixed standardized test meal that was high in fat. The test meal consisted of croissants, butter, high-fat ice-cream, chocolate and potato crisps with a macronutrient
composition per 2m$^2$ body surface area of 97 g fat, 124 g carbohydrate and 1450 kcal (MacEneaney et al., 2009). Body surface area (BSA) was derived using Haycock equation which was validated for infants, children and adults (Haycock et al., 1978). Further samples of blood were obtained at 60, 120, 240, 300 and 360 minutes after ingesting the meal. Subjects rested quietly during this period.

**Blood sampling**

Blood samples were collected before the test meal was ingested and five times hourly in the postprandial period. Subjects rested quietly during this period. Blood was collected using standard blood collecting sets and drawn into sterile tubes (EDTA). Serum was allowed to clot at room temperature for 20 minutes. Samples were then centrifuged at 1500g for 15 minutes, and the serum was removed before being frozen at -70C. Glucose levels were measured using enzymatic methods (Cobas Integra 800; Roche, Switzerland). The levels of other biochemical markers, serum TAG, total cholesterol, HDL and LDL, were also measured by enzymatic methods (Cobas Integra 800; Roche, Switzerland). Serum insulin was determined using standard fluoroimmunoassays (AutoDELFIA, Finland). Serum CRP was measured using competitive immunoassay (Roche Diagnostics, Germany).

**Statistical Analysis**

Data were analysed using SPSS version 17 (SPSS Inc., Chicago, USA). Two-way ANOVA with repeated measures was used to investigate differences between trials and across time for the following dependent variables: TAG, glucose, insulin, CRP, total
cholesterol, LDL and HDL. Area under curve (AUC) for TAG, glucose and insulin were calculated using the trapezoid method. Significant interactions revealed by ANOVA and AUC were followed up using Bonferroni adjusted t-tests. Statistical significance was accepted at $P \leq 0.05$. Data are presented as mean (SD).

6.3. Results:

Triacylglycerols

Triacylglycerol concentrations increased following the meal (time effect, $P < 0.05$) and were greater in the rest trial than the exercise trial ($P < 0.05$) (Figure 6.1). TAG AUC for the rest trial was greater than TAG AUC for the exercise trial ($P < 0.05$) (Figure 6.2). There was a significant interaction effect, and post hoc analysis showed that TAG concentrations were significantly higher in the rest trial than the exercise trial 120 and 180 minutes after consuming the test meal.
Figure 6.1: Mean (SD) TAG in the postprandial period following rest or exercise on the day before. *Significant difference between rest and exercise trials ($P < 0.05$).

Figure 6.2: Mean (SD) TAG AUC in the postprandial period during rest and exercise trials. *Different from exercise trial ($P < 0.05$).
**Insulin**

Insulin concentrations increased following the meal (time effect, $P < 0.05$) and were significantly greater in the rest trial than the exercise trial ($P < 0.05$) (Figure 6.3). Insulin AUC for the rest trial was greater than Insulin AUC for the exercise trial ($P < 0.05$) (Figure 6.4). There was a significant interaction effect, and *post hoc* analysis showed that insulin concentrations were significantly higher in the rest trial than the exercise trial 60, 120 and 180 minutes after consuming the test meal but lower at 360 minutes.

![Graph showing insulin levels over time](image)

Figure 6.3: Mean (SD) insulin in the postprandial period following rest or exercise on the day before. *Significant difference between rest and exercise trials ($P < 0.05$).
Figure 6.4: Mean (SD) insulin AUC in the postprandial period during rest and exercise trials. *Different from exercise trial \((P < 0.05)\).

**Glucose**

Glucose concentrations increased after the meal (time effect, \(P < 0.05\)) and were significantly greater in the rest trial than the exercise trial \((P < 0.05)\) (Figure 6.5). Glucose AUC for the rest trial was greater than glucose AUC for the exercise trial \((P < 0.05)\) (Figure 6.6). There was a significant interaction effect, and *post hoc* analysis showed that glucose concentrations were significantly higher in the rest trial than the exercise trial 120 and 180 minutes after consuming the meal.
Figure 6.5: Mean (SD) glucose concentrations in the postprandial period following rest or exercise on the day before *significant difference between rest and exercise trials ($P < 0.05$).

Figure 6.6: Mean (SD) glucose AUC in the postprandial period during rest and exercise trials. *Different from exercise trial ($P < 0.05$).
Cholesterol

Table 6.1: Effect of acute exercise on cholesterol

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rest</th>
<th></th>
<th></th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
<td>6h</td>
<td>0h</td>
<td>6h</td>
</tr>
<tr>
<td>Total Cholesterol(mmol.L(^{-1}))</td>
<td>3.0 (.7)</td>
<td>3.3 (.9(^*))</td>
<td>2.8 (.6)</td>
<td>3.1 (.7)</td>
</tr>
<tr>
<td>LDL-Cholesterol(mmol.L(^{-1}))</td>
<td>1.5 (.8)</td>
<td>-</td>
<td>1.7 (.5)</td>
<td>-</td>
</tr>
<tr>
<td>HDL-Cholesterol(mmol.L(^{-1}))</td>
<td>1.2 (.5)</td>
<td>1.3 (.2)</td>
<td>1.0 (.3)</td>
<td>1.1 (.1(^*))</td>
</tr>
<tr>
<td>CRP(mg.L(^{-1}))</td>
<td>6.5 (.9)</td>
<td>6.3 (.8)</td>
<td>5.7 (.5)</td>
<td>5.5 (.3)</td>
</tr>
</tbody>
</table>

Values are Mean (SD). *Significant difference between rest and exercise trials (\(P < 0.05\)).

6.4 Discussion:

The present study was designed to investigate the effect of consuming a high-fat mixed meal on postprandial TAG concentrations in Omani children with SCD and the effect of a single bout of exercise on this TAG response. The main findings were that a high-fat meal elicited an increase in plasma TAG concentrations and that the postprandial TAG, insulin and glucose responses were lower when exercise was performed the day before the meal, as has been reported for healthy children (Barrett et al., 2007; Tolfrey et al., 2008; Tolfrey et al., 2012). There was no difference in total cholesterol response between the exercise trial and the rest trial six hours after the meal.

Postprandial TAG is of great interest as an indicator for cardiovascular disease. The rise in plasma concentrations of triacylglycerol-rich lipoproteins following the consumption of a high-fat meal is considered to be a better marker of future
cardiovascular disease than fasting TAG alone (Su et al., 2009). Recently, the American Heart Association pointed to the need to design strategies aimed at primary prevention of atherosclerotic cardiovascular disease in childhood (Hickman et al., 1998 & Williams et al., 2002). In SCD, high serum TAG has also been correlated with impaired endothelial function, increase haemolysis, anaemia, inflammation and painful crisis (Belcher et al., 1999; Buchowski et al., 2007; Erasmus et al., 1990; Seixas et al., 2010; Zorca et al., 2010). To our knowledge, this is the first study that has assessed the effect of exercise on postprandial TAG in children with SCD, therefore the main findings of a reduction in plasma TAG concentrations in SCD children as a result of a single bout of exercise are novel.

Physical activity is known as effective tool for lowering postprandial TAG concentrations (Miyashita et al., 2008). A single bout of low- and moderate-exercise intensity was found to lower the postprandial TAG in healthy adults (Pfeiffer et al., 2005 & Tsetsonis & Hardman, 1996). Murphy et al., (2000) and Miyashita et al. (2008) reported that 30 minutes of accumulated brisk walking in one session reduces postprandial plasma TAG concentrations and increases fat oxidation. In addition, several studies that examined the influence of exercise on postprandial TAG in adolescent boys reported a decrease in postprandial TAG in response to exercise (Barrett et al., 2007; MacEneaney et al., 2009; Tolfrey et al., 2008; Tolfrey et al., 2012). The present study reported similar results, indicating that postprandial TAG concentration was higher in the rest trial than in the exercise trial. Higher TAG concentration 120 and 180 minutes after the meal in the rest trial suggested a slower clearance of TAG, compared to the exercise
trial. The mechanism responsible for the slower rate of TAG clearance following rest is not fully understood. One possible explanation for the faster TAG clearance in the exercise trial is an increase in the activity of LPL, a major enzyme in the TAG metabolism pathway (Gill & Hardman, 2003). It has been reported that adults who exhibit an increase in the activity of muscle or plasma LPL after a single bout of moderate exercise also exhibit a simultaneous reduction in fasting and postprandial TAG concentrations (Gill & Hardman, 2003; Herd et al., 2001). Both Herd et al. (2001) and Gill & Hardman (2003) suggested that although the increased LPL activity probably plays a role in the reduction of TAG, it is likely that other mechanisms such as increased activity of adipose LPL or reduced liver secretion of very low density lipoprotein (VLDL) are also involved. However, the purpose of the present study was not to investigate the possible mechanisms responsible for TAG metabolism.

Concentrations of insulin and glucose were also higher at certain time points in the rest trial than the control trial, with the AUC insulin also significantly greater in the rest trial. These findings demonstrate that the carbohydrates in the mixed meal provoked an insulin response. These results disagree with those found by MacEneaney et al. (2009), who reported no difference in insulin response to exercise in healthy adolescent boys. The insulin response is important in that insulin performs a significant function in regulating plasma TAG concentration through down-regulating LPL activity in skeletal muscle (Herd et al., 2001). It has been demonstrated that in adult humans, insulin decreases skeletal muscle LPL (Kiens et al., 1989). Thus, the greater rise in insulin observed in rest trial might, to some extent, explain the slower rate of TAG clearance.
Fasting total cholesterol concentrations have been reported to be lower in SCD patients than healthy controls (El Hazmi et al., 1987; Nagajothi & Sanmugarajah, 2005; Nnodim et al., 2012; Rahimi et al., 2006). Similar results were found in Nigerian boys and girls (Shores et al., 2003). The results of the present study are in agreement with those reported by Shores et al. (2003), as the mean baseline total cholesterol values were lower in this study population, compared to those reported for healthy adolescent boys (Barrett et al., 2007; MacEneaney et al., 2009). In response to exercise, MacEneaney et al. (2009) reported that total cholesterol concentrations were higher six hours after consuming the meal in the rest trial, compared to the exercise trial. In the present study, there was no difference in total cholesterol response between the two trials.

Postprandial TAG was lowered with the exercise utilised in this study setting, suggesting well-being advantages for this population. The lowering of postprandial TAG concentrations in individuals with SCD via exercise is likely to have beneficial effects in reducing pain crisis episodes and disease complications due to the reduced haemolysis, endothelial activation and inflammation described earlier (Belcher et al., 1999; Seixas et al., 2010; Zorca et al., 2010). An elevated circulating level of CRP is indicated to be a marker of vascular endothelial dysfunction (Cleland et al., 2000; Pasceri et al., 2000) as the accumulating evidence suggests that CRP induces expression of IL-6 and mediates LDL cholesterol uptake by endothelial macrophages (Libby, 2002; Pasceri et al., 2000; Ridker et al., 2003; Yeh, 2004). Increase in fasting and postprandial TAG has been indicated to be associated with higher fasting and postprandial CRP levels (Lanes et al., 2004). In the present study, there was no significant change in CRP concentrations six
hours after consuming the meal in both trials. Elevated TAG concentrations are associated with increased haemolytic markers, activation of the endothelium and inflammatory responses leading to higher chances of vaso-occlusive and inflammatory events (Zorca et al., 2010). Triacylglycerol elevation is also associated with increased acetylcholine release leading to reduced blood flow to tissue (Zorca et al., 2010). Therefore, any reduction in TAG concentrations is likely to have a beneficial impact for children with SCD. The net effect of reduction in TAG, insulin, and total cholesterol concentration resulting from mild- to moderate-exercise bouts may give a considerable lowering of disease complications and enhance the quality of life in the SCD population if exercise is repeated on a regular basis.

In conclusion, a single bout of exercise on the day prior to consumption of a high-fat meal reduces postprandial TAG concentrations in SCD children in a similar way to that which has been reported in normal healthy children. Most of the literature has investigated the effect of exercise on postprandial TAG in adults, and only a few studies have investigated this response in children. To our knowledge, this is the first attempt to examine postprandial TAG and exercise in SCD children. The findings of the present study support those already reported in normal healthy children and provide a new exploration of the value of exercise in reducing postprandial TAG. However, further investigations are required for a better understanding of the effects of regular exercise on postprandial TAG concentrations and the possible longer-term benefits to individuals with SCD.
7. GENERAL DISCUSSION

7.1 Discussion

Exercise is important in children’s and adolescents’ lifestyle and well-being. Studies in health and exercise have demonstrated a possible beneficial effect of exercise in SCD patients. A physically active lifestyle has a positive effect in decreasing endothelial activation and limiting the risk of vascular adhesion events in SCD patients (Aufradet et al., 2010). In addition, physical activity reduces vascular impairment associated with oxidative stress (Chirico et al., 2012) and improves the microcirculatory blood flow in patients with SCD (Waltz et al., 2012). Barbeau et al. (2001) suggested that regular exercise at moderate intensity could decrease the risk of inflammation in SCD, which enhances vasodilatation and thus decreases the risk for vaso-occlusive crisis.

Moderate exercise eliciting 50% of maximal aerobic power of 20 minutes duration has been proposed as a safe and feasible therapy to enhance and improve the quality of life of patients with SCD (Balayssac-Syransy et al., 2011) as it has been reported that exercise therapy may contribute to a reduction in the length of hospitalisation (Alcorn et al., 1984), decrease in pain and significant improvement of respiratory muscles strength (Tinti et al., 2010) in patients with SCD. The current thesis work was done to compare SCD and normal healthy children’s physical fitness and cardiovascular responses to exercise and to estimate whether exercise has beneficial effect on postprandial lipid metabolism in SCD children.
Sickle cell disease is defined as an abnormality of Hb through the mutation of normal HbAA into HbSS. Although SCD represents one of the most common haemoglobinopathies in Oman, literature related to the body composition, physical activity and health status of this population was limited. Therefore, the first study presented in this thesis aimed to assess general markers of physical fitness in children with SCD and SCT and compare them to normal healthy children. The second study was designed to investigate the cardiovascular responses to exercise in this population (SCD and SCT) and compare them to normal healthy children from the same age ranges. Given the role played by inflammation in the clinical course of the disease, the third study was designed to investigate the effect of exercise on IL-6, a marker of inflammation, in SCD children and compare its responses to normal, age-matched, normal healthy children from Oman. The fourth study was designed to estimate the effect of one bout of exercise on postprandial lipaemia in SCD children.

Owing to the presence of Hb SS and its physiological effect on its carrier’s haemostatic balance and blood function, and given the influence of exercise on the physiological characteristics of Hb, it was expected that children with SCD would show poor physiological responses, such as lower cardiopulmonary fitness demonstrated as lower VO\textsubscript{2} max and exaggerated blood pressure, heart rate and lactate responses, that will limit their tolerance to exercise.

It has been reported that children with SCD in general are shorter and lighter, have a higher proportion of their body mass as fat and have late skeletal maturation,
compared to normal healthy children (Barden et al., 2002; Platt et al., 1984; Zemel et al., 2007). The main anthropometric findings of this thesis work was similar to those reported in the literature, indicating that SCD children had smaller stature, lower body mass, a greater proportion of their body mass as fat and late skeletal maturation. Previous studies in Omani children with SCD have reported similar anthropometric results (Moheeb et al., 2007; Wali & Moheeb, 2011). It is still not clear whether these are causes or manifestations of the lower fitness, as the present work showed that children with SCD carried out less physical activity than the normal healthy children. However, it has been reported that adolescents with SCD perform less physical activity related to their reduced oxygen-carrying capacity of blood. Factors contributing to the reduced oxygen-carrying capacity in this population include lower Hb level, vascular impairment, and functional cardiac and pulmonary changes (Alpert et al., 1984; Braden et al., 1996; Castro et al., 1994) associated with chronic anaemia (Buchowski et al., 2002).

Sickle cell disease subjects have altered haematological indices. The present work showed that Hb, RBC, and Hct values were lower in SCD children. These parameters are the main components that determine the blood’s capacity to deliver oxygen to the body, thus, any abnormalities in these components will be manifested as improper oxygen delivery to the tissues. These results were reflected in the multi-stage fitness test, which indicated that SCD children exhibited lower estimated VO$_2$max than SCT and normal healthy children. In addition, SCD children had higher heart rate and lactate responses to five minutes of treadmill walking. These results support those reported by Pianosi et al. (1991) and Braden et al. (1996), who reported lower VO$_2$max in SCD children. In
addition, Callahan et al. (2002) reported abnormal VO$_2$max and heart rate responses to exercise in adult women with SCD. During exercise, there is an increase body demand for oxygen; in the present work, the higher lactate response suggests a shift to anaerobic metabolism and the early increase in the heart rate and blood pressure suggests a compensatory mechanism in response to the lower oxygen-carrying capacity of blood related to lower Hb concentrations. These physiological parameters response might explain the limited exercise tolerance reported by SCD children in multi-stage fitness test as they reached exhaustion earlier than SCT and normal healthy children.

The haematological measurements in the present work indicate that children with SCD have a higher baseline WBC, indicative of a chronic inflammatory state. It has been indicated that inflammation and vaso-occlusive events are among the most significant complications in SCD. Given the role played by IL-6 and lipid profile in inflammation and in the occurrence of vaso-occlusive events (Pathare et al., 2003; Zorca et al., 2010), the effect of postprandial lipaemia demonstrated in the present work indicates that exercise might give a beneficial effect in this population.

Previous research has suggested that IL-6 concentrations are elevated in response to exercise, as it is the first cytokine to be released into the circulation (Pedersen & Febbraio, 2005). Thus, it was expected that both the SCD and normal healthy group would have an IL-6 response to a single bout of exercise and that there would be a difference in the IL-6 responses between the two groups. The results of this work indicated that there was no significant change in IL-6 concentrations in response to
exercise in either normal healthy or SCD children. SCD children reported higher mean baseline IL-6 concentrations than normal healthy children, and these remained higher throughout the test. To our knowledge, only one study has investigated the effect of exercise in IL-6 in a population with SCD (Barbeau et al., 2001). In the present study, SCD children’s response was similar to those reported by Barbeau et al. (2001), who reported no significant change in IL-6 concentration in SCD subjects in response to exercise and after 24 hours of a single bout of exercise. However, Barbeau et al.’s (2001) work on the responses of inflammatory and vasoactive markers to exercise in adults with SCD suggests that regular moderate-intensity exercise might decrease the risk of inflammation in this population as exercise increased NO concentrations. Nitric oxide induces vasodilatation and therefore reduces the risk for vaso-occlusive crisis.

Another important contributor to inflammation in SCD is TAG. Elevated serum TAG in SCD patients has been associated with haemolysis, inflammation and endothelial activation contributing to increased risk of vascular dysfunction, increased rate of haemolysis (Seixas et al., 2010; Zorca et al., 2010) and painful crisis (Buchowski et al., 2007; Erasmus et al., 1990). Therefore, a better understanding of TAG response to exercise in this population is important. It was expected that the serum TAG concentrations would increase after a high-fat meal and that the postprandial TAG would be lower after exercise in individuals with SCD. The findings met those expected, as postprandial TAG concentrations were higher in the rest trial than in the exercise trial, suggesting a slower clearance of TAG in the rest trial. In addition, postprandial
insulinemia tended to be lower following exercise. The effects of the exercise trial suggest an alteration of the lipid profile that might have beneficial outcomes for this population. Previous research has indicated that higher blood TAG and cholesterol concentrations are associated with lower blood flow and increased endothelial damage in SCD subjects (Belcher et al., 1999; Zorca et al., 2010). Exercise has consistently been shown to reduce postprandial TAG in healthy young boys (Barrett et al., 2007; Tolfrey et al., 2008; Tolfrey et al., 2012). To our knowledge, no research has been published examining the effect of exercise on postprandial TAG in children with SCD. The mechanism behind the faster clearance of TAG in response to exercise is still not clear, although several studies have suggested that an increase in the activity of skeletal muscle LPL might be a possible mechanism, as an increase in the enzyme activity simultaneous with a decrease in serum TAG concentrations has been indicated (Gill & Hardman, 2003; Herd et al., 2001). Other studies have suggested a possible contribution of insulin in the slower clearance of postprandial TAG at rest, through lowering the activity of LPL (Herd et al., 2001; Kiens et al., 1989). Thus, the insulin response observed in the present study might contribute to the higher TAG concentrations in the rest trial. The present work adds to these findings to demonstrate that an acute bout of exercise attenuates postprandial TAG in children with SCD, which implies that there might be health benefits to this population.

Limitations to the studies in this thesis may include the absence of a reliable and valid measure of physical activity and maturation, such as the average physical activity of the individual and skeletal maturation for the Omani population, which are important
variables when investigating the role of exercise in children. In addition, this makes it difficult to compare the results with the Western population, since most of the testing and measurement techniques used in this study have been validated for Western children. In addition, the absence of reliable and valid haematological indices for the Omani population makes it difficult to speculate whether the data of this study can be standardised to the whole population. This work would have benefit from measuring more markers that have been associated with vascular endothelial dysfunction such as NO, TNF-α and ET-1 as these markers will help evaluate the overall endothelial dysfunction in these children. Another method would be to study the inflammatory markers response in the postprandial state. The lack of research supporting laboratories in Oman and the need to ship blood samples to UK limited the ability to investigate such markers. There is also a limit to the generalisability of the results to females. It would have been difficult to study females throughout this thesis, owing to cultural obstacles. The lack of sexual maturation measurements may also have influenced the results as research has documented the impact of sexual maturation on physical fitness, although estimating skeletal maturation might be considered more relevant in this regard anyway.

7.2 Future directions

Research investigating SCD disease status and manifestation in Oman is limited; therefore, the role for exercise in children with SCD in Oman represents a rich area for research.
Several studies have indicated that SCD patients show elevated blood viscosity (Nebor et al., 2011; Platt et al., 1991), increased soluble adhesion molecules, abnormal RBC rheological properties (Alexy et al., 2010; Awodu et al., 2009; Ballas & Mohandas, 2004; Bowers et al., 2011), tendency of RBCs for adherence to the vascular endothelium (Hebbel, 1997; Kaul & Nagel, 1993) and marked endothelial dysfunction (Kato et al., 2005; Lin et al., 2005). It has been documented that exercise and physical activity may promote vaso-occlusive episodes in subjects with SCD through the induction of several changes, including increase in circulating cytokines, adhesion molecules and reactive oxygen species (Makis et al., 2000), and endothelial dysfunction related to decreased NO (Kato et al., 2005; Lin et al., 2005). These alterations may stimulate the sickling of RBCs leading to microvascular occlusion (Makis et al., 2000; Moheeb et al., 2007). However, most of these studies had investigated the effect of acute bouts of moderate intensity exercise while no study has been performed to investigate the effect of a moderate intensity training program on SCD patients. It has been recommended that exercise intensity for SCD subjects should be equal to or less than the anaerobic threshold (AT) of 35–60% VO$_2$max (Anthe et al., 2007; Callahan et al., 2002). Waltz et al. (2012) suggested that an acute bout of exercise at the indicated AT for this population could be safe and may improve haemorhaeological properties in SCD subjects. In addition, it has been suggested that exercise rehabilitation program of suitable intensity is unlikely to induce harmful metabolic changes and may be advantageous in many chronic diseases (Meyer et al., 2005; Myers, 2005; Palange et al., 2007). Therefore, studying the responses of children with SCD to a training programme including moderate-intensity
exercise would greatly add to our understanding of how regular exercise might affect the health of SCD children.

7.3 Conclusion

This work represents a new approach in the field of exercise and SCD as it studies some important biological markers that have been related to the disease severity in children. We could not find any published research regarding the effect of exercise on postprandial lipaemia in Middle Eastern children with SCD. Taking into consideration the fact that SCD is one of the most common haematological disorders in the Middle East, the findings in this thesis may contribute to an important educational tool to raise the public awareness regarding exercise and its potential to improve the health status of SCD children. The results indicate that SCD children have lower fitness, compared with normal healthy children. The lower fitness level observed might be a function of impaired growth and maturation or lower physical activity levels reported in this population. In addition, while mild- to moderate-exercise did not induce alterations in inflammatory IL-6 concentration, it lowered postprandial lipaemia, which is considered to reflect a positive effect of exercise.

To our knowledge, this is the first work to underline the possible role of exercise in postprandial lipaemia in children with SCD. Therefore, the results of exercise-induced lowering of TAG concentration in this population are novel and might be a starting point in redefining perceptions of the importance of regular exercise in children with SCD.
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Appendix 1: Physical activity readiness questionnaire LTEQ

Godin Leisure-Time Exercise Questionnaire

INSTRUCTIONS

In this excerpt from the Godin Leisure-Time Exercise Questionnaire, the individual is asked to complete a self-explanatory, brief four-item query of usual leisure-time exercise habits.

CALCULATIONS

For the first question, weekly frequencies of strenuous, moderate, and light activities are multiplied by nine, five, and three, respectively. Total weekly leisure activity is calculated in arbitrary units by summing the products of the separate components, as shown in the following formula:

Weekly leisure activity score = (9 × Strenuous) + (5 × Moderate) + (3 × Light)

The second question is used to calculate the frequency of weekly leisure-time activities pursued “long enough to work up a sweat” (see questionnaire).
EXAMPLE

Strenuous = 3 times/wk

Moderate = 6 times/wk

Light = 14 times/wk

Total leisure activity score = \((9 \times 3) + (5 \times 6) + (3 \times 14)\) = \(27 + 30 + 42 = 99\)

Godin Leisure-Time Exercise Questionnaire

1. During a typical 7-Day period (a week), how many times on average do you do the following kinds of exercise for more than 15 minutes during your free time (write on each line the appropriate number).

*Times Per Week*

a) **STRENUOUS EXERCISE**
   (HEART BEATS RAPIDLY)

   (e.g., running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)
b) MODERATE EXERCISE
   (NOT EXHAUSTING)  
   (e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)

c) MILD EXERCISE
   (MINIMAL EFFORT)  
   (e.g., yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking)

2. During a typical 7-Day period (a week), in your leisure time, how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)?

<table>
<thead>
<tr>
<th></th>
<th>OFTEN</th>
<th>SOMETIMES</th>
<th>NEVER/RARELY</th>
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<tr>
<td>1.</td>
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</table>
**Appendix 2: Results form**

Date: ..............

Name ...............................................................................................................................

Date of birth ............................................

Dad height.................

Mom height.................

**Pre-test**

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<table>
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<tbody>
<tr>
<td>Resting blood pressure</td>
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<td></td>
<td></td>
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<tr>
<td>Resting heart rate</td>
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<p>| | |</p>
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<tbody>
<tr>
<td>Height (cm)</td>
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<tr>
<td>Weight (kg)</td>
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<tr>
<td>BMI (kg/m²)</td>
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</table>
## Body fat

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<tr>
<th>Skinfold</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Average</th>
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</thead>
<tbody>
<tr>
<td>Triceps</td>
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<tr>
<td>Subscapular</td>
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<tr>
<td>Fat %</td>
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</tbody>
</table>
Appendix 3: Consent form

Dear Parent

I am an Omani postgraduate student under the direction of Dr Keith Stokes in the Sports and Exercise Science Group of School for Health at the University of Bath (United Kingdom). I am conducting a research project to investigate the effect of exercise in selected inflammatory mediators in children with SCD/SCT and compared with normal children.

Your child’s participation will involve participating in physical fitness tests such as treadmill walking test and sum of body composition. Tests will include taking venous blood samples taking for the subjects pre- post exercise. All measurements will take place at cardiology unit at Sur hospital under supervision of the researcher, doctor and nurse at all occasions. Your child’s participation in this study is voluntary. If you or your child decided not to participate or to withdraw from the study at anytime, there will be no penalty (for example it will have no effect on your child’s grade at school).

Although there may be no direct benefit to your child, the wider benefit of your child’s participation is in helping us to understand the impact of the SCD on exercise performance and the inflammatory responses of the body.

If you have any questions concerning this research study or your child’s participation in the study, please contact me on …
Mahfoodha Al. Kitani

92662660

I give/ I don’t give consent for my child ……………………………….to participate in the above study.

Parent’s Name: …………………………………………………

Parent’s Signature: ………………………………………….Date
بيبسي إجراء البحث بهدف التعرف على تأثير الرياضة على بعض المكونات المناعية لدى الأطفال المصابين بالأنيميا والأشخاص بالأطفال الأصحاء من نفس الأعمار. وسيشمل إجراء البحث المشي على جهاز التردم لفترة من 8-21 دقيقة ومن ثم يتم أخذ العينات القبلية والبعدية للاختبار مع أخذ بعض القياسات لمكونات الجسم (سمك الدهن).

سيتم إجراء الاختبار والقياسات في وحدة القلب في المستشفى تحت إشراف الباحثة وتواجد الدكتور المشرف وممرضة مع أخذ الاختبارات مسبقاً للعينة التي سوف تشارك في البحث ضمانا لسلامة المشاركين في البحث.

هذا ونحيطكم علمًا أن المشاركة في البحث (اختيارية) سواء للمرضى أو الأصحاء ولن يكون لها أي تأثير دراسيًا (سيتم مخاطبة المدرسة للعينات التي تحتاج إلى موافقة مدير المدرسة) وتوفير وسائل النقل لهم أيضاً. كما يمكن للمشارك أن ينسحب في أي وقت أراد أثناء إجراء الاختبارات.

استفادة الأطفال المشاركين في البحث تتلخص في معرفة الحالة العامة الصحية والرياضية لديهم وتسهيل النتائج على الوقوف على مدى تأثير الرياضة المنخفضة الشدة (إيجابياً أو سلبياً) على بعض المكونات الفسيولوجية للأطفال المرضى والأصحاء مما يساعد على زيادة المعلومات التي قد تساعد على فهم التغييرات التي قد تلعبها الرياضة في جسم الطفل.

هذا ونستقبل فائق إحترامي، ونرجوا منكم عدم التردد في الاتصال بالباحثة للسؤال في أي شيء يتعلق بالبحث.
الباحثة: محفوظة الكيتاني

جامعة السلطان قابوس - جامعة باث

92662660

توقيع ولي الأمر:

موافق على اشتراك أبني، ابنتي في البحث أعلاه

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